Universidade de Aveiro Departamento de Biologia
Ano 2016

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Desenvolvimento de indicadores ecológicos de alterações climáticas baseados na diversidade funcional de líquenes

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia e Ecologia das Alterações Globais, realizada sob a orientação científica do Doutor Amadeu M. V. M. Soares, Professor Catedrático do Departamento de Biologia da Universidade de Aveiro e da Doutora Cristina Branquinho, Investigadora Associada de Ecologia e Ciências Ambientais da Faculdade de Ciências da Universidade de Lisboa .

Apoio financeiro da FCT e do FSE no âmbito do III Quadro Comunitário de Apoio financeiro da FCT através da bolsa doutoral SFRH/BD/51419/2011.

o júri

Doutora Maria Herminia Deulonder Correia Amado Laurel, Professora Catedrática, Universidade de Aveiro

Doutor Paolo Giordani, Investigador, Università degli Studi di Gennova, Itália

Doutor João António de Almeida Serôdio, Professor Auxiliar com Agregação, Universidade de Aveiro

Doutor Artur Jorge da Costa Peixoto Alves, Investigador Principal, Universidade de Aveiro

Doutor Nuno Miguel dos Santos Ferrand de Almeida, Professor Catedrático, Universidade do Porto

Doutora Cristina Maria Branquinho Fernandes, Investigadora Principal, Universidade de Lisboa

Doutor Amadeu Mortágua Velho da Maia Soares, Professor Catedrático, Universidade de Aveiro

to my mom and my dad

agradecimentos

To my supervisors, I don't think I could have had a better combination than this! Thank you for the all the inspiration, the support, for believing in me, and for giving me the opportunity to do what I love! None of this would have been possible without you.

I have also to thank all the amazing people that help me along this way. To Pot's team from Bangkok University, thank you for the field work support in Khao Yai National Park. To the Instituto Ecológica team in Palmas for receiving me there and facilitating all the field work. To the group from Universidade Federal do Tocantins that adopted me and made my stay there so much better! To Marissónia and Roberto from IECOS, for having me there and helping me on field work. Goyo and Isabel, it was a pleasure to be there, thank you both and your team in Madrid, for having me there and for the field work, data and discussions. To Paolo from Genoa, it is always a pleasure to work and discuss science with you, and always so much fun! To Linda, first for inviting me to the International Internship program in the US Forest Service in Corvallis, for receiving so warmly, for coming here, for all the work and lastly, for all the English classes! Thanks to Amanda, my amazing wilderness mate, to Doug and to the inspiring Bruce. To Andre Aptroot for his help on tropical lichens identification. To Eng. Gama and Sr. Alfredo for all the support in field work at Castro Verde. And, of course, last but not at all least, to my team from Lisboa! After all, they are the ones having to put up with me everyday! Thanks to Pedro and Alice, my core dream team during this journey, this is also your work! To all the other eChanges and ESFEs that supported, inspired, held my hand, heard me screaming and laughing along this way. I can't name everyone, but this thesis has a bit of each one of you in it!

Travelling alone is not as much fun, as with good company. So I have to thank my two Doutoretes partners that started this adventure with me!

Thanks to all my friends! To my chuchus, Cris, Concos, Lau e Teresa. You're my cornerstone! And to Ana and Mel, the new travelling mojitas! To Teresa A., Sol and Lenita, my old time friends. Thanks to my Suhsi! My four-legged version, my shadow, my friend, that even with all my absences, likes me just as much. Many thanks to my family. To my brother and my sister in law. To my little prince Afonso, you make my life so much more colourful! Thank you all for putting up with me! For being always there for me. And to my nieces Laura and Luísa. Incredible that you're still able to remember my face!

Finally, thanks to you mom! The best mom in the world!

I feel so lucky that I got to live these four and a half years. It was a true pleasure to make this work, so I just hope you enjoy it as much as I do.

Everything in my life has a sound track, my thesis could not be an exception. Now sit back, relax and enjoy the music that inspired me along this way. Just click on the quavers or read the QR code!

palavras-chave atributos funcionais de resposta, grupos funcionais, espaço-pelo-tempo.

resumo

O clima global tem vindo a mudar ao longo das ultimas décadas e um conjunto de variáveis climáticas está a ser desenvolvido para monitorizar estas alterações climáticas. No entanto, isso não é insuficiente. É imperativo compreender e quantificar como o funcionamento dos ecossistemas é afetado e responde a estas alterações, e os indicadores ecológicos baseados na biodiversidade são uma das ferramentas para o fazer.

O objectivo deste trabalho foi desenvolver um indicador ecológico global e integrado baseado na diversidade funcional de líquenes, para avaliar os efeitos das alterações climáticas nos ecossistemas. Para isso usamos um gradiente climático no espaço, assumindo o espaço-pelo-tempo como proxy. Para ser global, desenvolvemos um enquadramento de análise conjunta dos dados das duas metodologias de amostragem de líquenes usadas à escala continental (EUA e EU). De seguida, mostramos que os atributos funcionais de líquenes relacionados com o tipo de fotobionte, forma de crescimento e tamanho respondem ao clima, nomeadamente à amplitude e variação sazonal da temperatura e precipitação e à humidade relativa média anual. No entanto, a resposta foi específica para cada grupo funcional (p.e. cianolíquenes) mostrando que os atributos funcionais no seu todo (p.e. tipo de fotobionte) não respondem universalmente às mesmas variáveis climáticas. De seguida, confirmamos que cada grupo funcional refletiu as variáveis climáticas subjacentes de forma previsível, validando o seu uso como indicadores ecológicos. Porque esta abordagem não foi limitada pela identidade das espécies, testamos a sua aplicação à escala global. Descobrimos que os pares grupo funcional/variável climática subjacente não podem ser aplicados à escala global; a resposta é específica de áreas com o mesmo factor climático limitante (p.e. água). Porque respondem ao factor climático mais limitante e este varia à medida que nos movemos no globo, a sua aplicação tem que ser especifica a áreas com os mesmos factores climáticos limitantes. Finalmente, tentamos compreender se o proxy espaço-pelo-tempo usado era válido. Os indicadores ecológicos previamente identificados ao longo de um gradiente climático espacial, foram os que responderam a mudanças climáticas ao longo de 15 anos e esta resposta foi sensível a mudanças subtis no clima, não detetadas pelas métricas clássicas de clima. Em conclusão, os líquenes são de facto excelentes indicadores ecológicos para avaliar os efeitos das alterações climáticas, com potencial de alerta precoce.

Organizações internacionais como as três convenções irmãs das Nações Unidas há muito exigem um conjunto de indicadores para avaliar os efeitos das alterações climáticas, que possam monitorizar continuamente. De hoje em diante, os líquenes podem ser incluídos neste conjunto e esperamos que este trabalho fomente o uso da diversidade funcional de líquenes como uma ferramenta para avaliar e seguir os efeitos das alterações climáticas nos ecossistemas do mundo inteiro.

keywords

response traits, functional groups, space-for-time.

abstract

Growing evidence shows us that climate has changed in the recent decades, and the scenario for the future will most likely worsen. A set of climate variables is being developed to monitor climate change, but this is not enough to keep track its effects on ecosystems. It's imperative to understand and quantify how ecosystems functioning are affected by and respond to these changes, and ecological indicators based on biodiversity metrics are one of the tools to do this.

The objective of this work was to develop a global lichen functional diversity integrated ecological indicator of the effects of climate change on ecosystems. For that, we used a climatic gradient in space assuming a space-for-time proxy. To be global, we developed a framework to jointly analyse data from the two methodologies currently used at the continental scale (US and EU). Afterwards, we found that lichen traits, main type of photobiont, growth form and size respond to climate, namely to the range and seasonal variation of temperature and precipitation, and to relative humidity. However, the response was specific for each functional group (e.g. cyanolichens) showing that traits (e.g. photobiont) as a whole do not respond universally to the same climate variables. Then, we confirmed that each functional group reflected the underlying climate variables in a predictable way, validating their use as ecological indicators of climate change. Once our approach was not limited by species identity, we tested its potential to be used at a global scale. We found that each couple of functional group and underlying climatic variable cannot be applied at the global scale; the response is specific to areas with the same climatic limiting factor (ex: water availability). Because lichen functional group-based indicators respond to the most limiting climate factors, and these vary as we move on the globe, its application must be specific to areas with similar climate limiting factors. Finally, we wanted to understand if the space-for-time proxy was valid. The ecological indicators previously found using a spatial approach for a specific climate were in fact those responding to climate shifts over fifteen years. Moreover, these indicators were able to respond to subtle shifts in climate no yet depicted by classic climate metrics. Thus lichens are in fact excellent ecological indicators to track the effects of climate change, and have the potential to be early warning.

International organizations such as the Unite Nations three sister conventions have long demanded for a set of ecological indicators to track the effects of climate change, that can be monitored continuously over time. From now on, lichens can be included in this set of ecological indicators and we expect that this work will foster lichen functional diversity use as a tool to track climate change worldwide.

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chapter one Introduction

global change drivers

Human activities in the Industrial Revolution triggered dramatic environmental changes, probably enough to push Earth out of its stable Holocene period over to a new Anthropocene era (Crutzen 2002). This generalized industrialization stimulated an increasing dependence on power generation for industry and urban needs (like transportation, or domestic heating), drastically raising pollutants levels in the atmosphere (Fig. 1, historical trends). Sulphur dioxide (SO_2) soon became a key air pollutant, originated mostly from coal and oil combustion in this industrializing world (Amann, Klimont & Wagner 2013). Its emissions caused a series of environmental problems like smog, acid rain and dry deposition (Stern 2006), all with adverse significant impacts on human health, food provision and ecosystems (IPCC 2001; WHO 2006; Amann, Klimont & Wagner 2013). The Industrial Revolution lead also to new forms of agriculture, more intensive and industrialized. By the end of the 19th century, crop production was unable to feed the increasing human population due to limiting biological nitrogen supply (Sutton *et al.* 2011). The industrial production of fertilizers ended this limitation, revolutionizing agriculture in the beginning of the 20th century (Sutton *et al.* 2011). Along with this progress, came severe environmental consequences and agriculture became a major source of nitrogen pollution, exacerbating the already existing loads of nitrogen arriving from fuel combustion (Sutton *et al.* 2011). After the emissions peak in the late 1970s, the adoption of measures to control industrial and urban pollutants emissions has successfully reduced their levels in the atmosphere (Fenger 2009). In the past two decades, Europe and North America SO_2 levels dropped more than two-

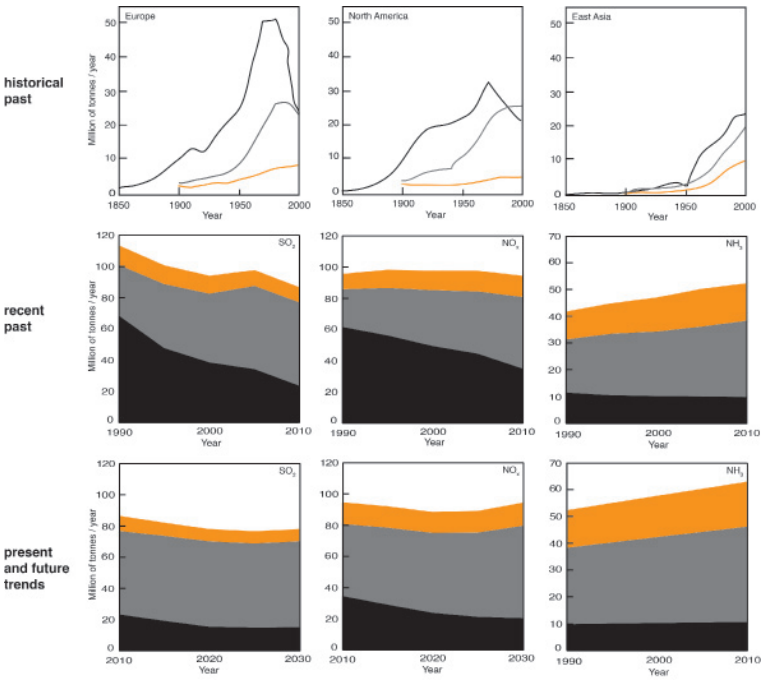

fig. 1. Historical, recent past and present and future trends of major atmospheric pollutants. In historical trends, black line represents SO_2, grey NO_x and orange NH_3, for Europe, North America and East Asia (adapted from http://forum.eionet.europa.eu/nrc-flis/library/consultation-countries/gmt-update-2013/gmt10/global-megatrend-10-increasing-environmental-pollution-load/10.2-status-and-trends-emissions). Recent past and present and future cumulative trends are shown for SO_2, NO_x and NH_3: N. America, Europe and Russia in black; Asia and Pacific in grey; and the rest of the world in orange (adapted from Amann 2013).

thirds as a result of enhanced energy efficiency, new fuel blends and end-of-pipe desulphurization in the energy sector (Vestreng *et al.* 2007; US Environ. Prot. Agency 2012; Tørseth *et al.* 2012). Asia, although with temporary peak increases compensating the declines in other world regions, was able to resume a decreasing trend, setting global SO_2 trends into a downturn (Amann, Klimont & Wagner 2013). Thenceforth, nitrogen pollution emerged as the major driver of change, surpassing SO_2 trends (Rockström *et al.* 2009), fig. 1).

This massive dependence of humans on fossil fuels likewise lead to a colossal increase in greenhouse gases emissions (GHGs) that, together with other major drivers of change like SO_2 and NO_x, set in motion a change in climate (IPCC 2007; IPCC 2014). In fact, it is beyond dispute that the climate system has changed in the recent decades, with impacts on natural and human systems across all continents (IPCC 2014). If current efforts to reduce nitrogen emissions are successful, climate change is expected to gain increased importance as a global change driver. It is not yet fully understood the pathway global environmental change will follow and what will be its outcome, but regarding climate, even if anthropogenic greenhouse gases emissions stop, warming and its associated impacts will continue far beyond the 21st century (IPCC 2014).

climate change and its consequences

Unlike pollution, climate change is usually addressed at a global scale. Pollution effects tend to occur at a local scale in the near term, and related problems or emissions control are generally addressed at a more local, national or regional scale (Amann, Klimont & Wagner 2013). The global nature of climate change is perhaps more clearly illustrated if we think that, as shown by human balloon flights, the air will take typically a week to fly halfway around the world (Karl & Trenberth 2003). GHGs emissions emitted locally over time, contribute to raise the overall atmospheric concentrations, changing climate at a global scale and causing borderless impacts across the globe (Karl & Trenberth 2003).

Global climate has unequivocally changed over the recent decades (IPCC 2014). Under all greenhouse gas emission scenarios for the 21st century, surface temperature is still forecasted to rise more than 1ºC. Also the frequency and duration of temperature extremes will rise at the global scale. Precipitation patterns will continue to change, although not uniformly. In the particular case of Europe and North America, with ongoing warming trends, both continents mid-latitude land masses will experience frequenter and intenser precipitation extremes. A worrying decrease in mean annual precipitation is expected over its mid-latitude dry regions,

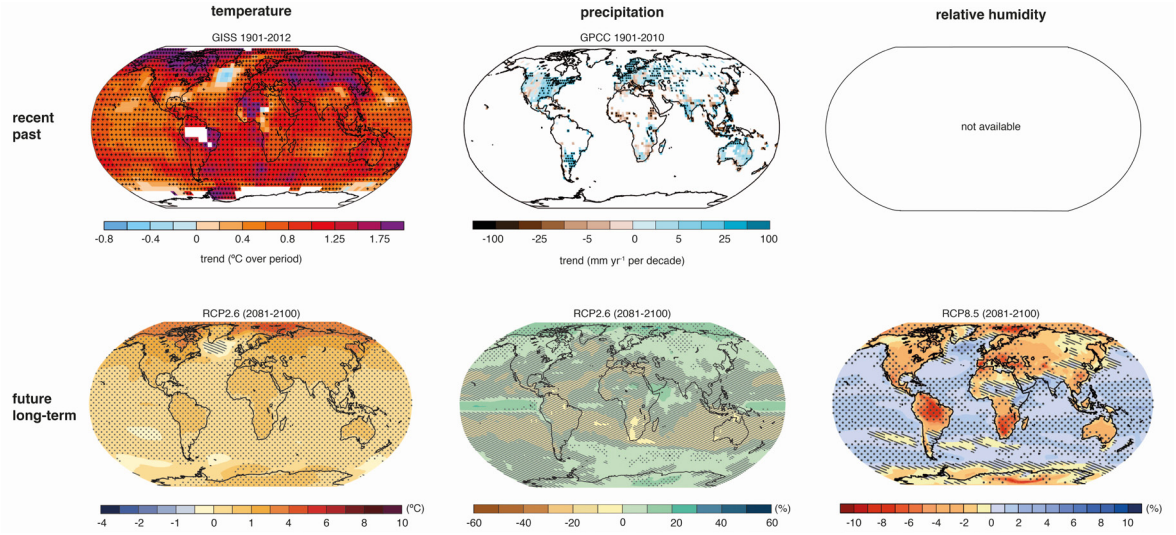

fig. 1. Past climate trends and future projections of temperature, precipitation and relative humidity. White areas indicate incomplete or missing data in past maps. Future long-term maps for temperature and precipitation show projection for the lowest CO_2 concentration scenario (RCP 2.6) and for the relative humidity only the highest concentration scenario (RCP 8.5) is available. Adapted from IPCC 2014.

particularly in southern Mediterranean Europe, where 30 % less precipitation is expected (Fig. 2). Conversely, mid-latitude wet regions will likely experience an increase in mean precipitation. Globally, near surface humidity will also change. Near surface specific humidity (roughly defined as ratio of water vapour mass to the total air mass) has increased since the 1970s (Hartmann *et al.* 2013) and is projected to continue to rise over the near future (Kirtman *et al.* 2013). On the other hand, near-surface relative humidity over most land areas (excepting Africa and India) will likely decrease in the near future (Kirtman *et al.* 2013) and over to end of the 21st century as a result of global warming (Collins *et al.* 2013). These unprecedented climate warming and precipitation pattern changes have already impacted natural and human systems and with the foresee changes the scenario will most likely worsen (IPCC, 2014).

The observed and expected worldwide social and ecological impacts of climate change raised a generalized concern, leading to the prioritization of tracking climate change effects on ecosystems by both scientific community and the international governmental sphere. Tracking and monitoring climate change is necessary to evaluate the impacts at the ecosystem level at a global scale and perhaps more importantly, to assess the global effects of the international policy and governance measures adopted in its response (Branquinho, Matos & Pinho 2015). Following this, the three sister UN conventions, United Nations Conventions on Biological Diversity (UNCBD), Climate Change (UNCCC) and Combatting Desertification (UNCCD) have long

demanded a set of globally applicable indicators. This is an essential step to globally assess the effects of global change drivers, and maintain an observation over time, in an easy-to-implement, routine, and consistent way.

Tracking climate change and making informed decisions on prevention, mitigation and adaptation strategies demands that climate observations are sustained consistently over time (GCOS 2004). To ensure long-term, uninterrupted, high quality observations of climate at the global scale, the United Nations Framework Convention on Climate Change (UNFCCC) created a Global Climate Observing System (GCOS 2004). When fully implemented, this GCOS will provide high quality data on a set of Essential Climate Variables (ECV) that were chosen (a process still ongoing and evolving according to technology and advances in research) to allow a better future impact assessment of climate change. This characterization of the state of global climate is fundamental to support attribution of causes, support better predictions at higher resolution and enable the characterization of extreme events to define risk assessment and vulnerabilities and accordingly adaptation measures (GCOS 2004). However, measuring drivers' change over time provides information on the state of the environment, but does not provide any information about its impact on ecosystems (Branquinho, Matos & Pinho 2015). It is obvious to assume that the predicted 2ºC increase in global temperature will not have the same effect on alpine and desert ecosystems. Evaluating the impacts of climate change on ecosystems demands more than just a measure of the climate variables. It is essential to have measures capable of describing climate change impacts on ecosystems, biodiversity, structure and functioning (Pereira *et al.* 2013).

ecological indicators & biodiversity metrics

Due to its complexity, it's extremely hard to quantify all ecosystem properties or entirety (Lindenmayer *et al.* 2015) in response to global change drivers. If the temporal and spatial scale at which the information is needed is contemplated, the task is even more troublesome due to logistic and cost constrains (Lindenmayer *et al.* 2015). Ecological indicators are measurable characteristics of the structure, composition and functioning of ecosystems (Niemi & McDonald 2004). They can be efficiently used to measure and describe the effects of environmental change on ecosystems structure and functioning in a cost-effective manner, providing simple and representative information that can then be communicated to environmental stakeholders for decision-making (Branquinho, Matos & Pinho 2015). Biodiversity is the basis of ecosystems integrity and human livelihood, as it affects ecosystems functioning and their ability to provide the goods and services (e.g. availability of fresh water, food and fuel)

that sustain society (Cardinale *et al.* 2012). Hence, biodiversity based ecological indicators are within the best to measure the effects of climate change and the other global change drivers on ecosystems.

During many years, species richness was the metric of choice to quantify ecosystems change in response to environmental drivers. Although this metric is fundamental to track the irreversible component of loss related to global change drivers, this index performs poorly when measuring global change drivers. Despite the expansion in the number of threatened species (Ricketts *et al.* 2005) and the projected increase for the future (Thomas *et al.* 2004), at the local scale, global trends remain unresponsive (Dornelas *et al.* 2014). This is likely related to the spatial scale addressed (McGill *et al.* 2015) and/or to the time lag of its response to climate change (or other global change drivers) (Menéndez *et al.* 2006). Furthermore, this metric is incapable of showing the component of change in biodiversity patterns. Compositional shifts have been observed over time in response to global change (Dornelas *et al.* 2014), and if only species-related metrics are used, species redundancy in the ecosystems will not be accounted for when compositional shifts occur. Moreover, comparison at larger geographical scales may be impaired due to species limited distributions if taxonomic diversity metrics are used. Functional diversity is a better indicator to quantify ecosystem functionality in response to global change drivers (Díaz & Cabido 1997; Díaz *et al.* 2007; Suding *et al.* 2008; Lavorel *et al.* 2011; Mouillot *et al.* 2012). This functional trait approach is able to quantify compositional shifts accounting with species redundancy and has the potential to be both universal and applicable at broad spatial scales, because it is not linked to species per se. A functional trait is a characteristic of an organism that is relevant to its response to the environment and/or its effects on ecosystem functioning. The range or value of traits in a given ecosystem are, by definition, a measure of functional diversity (Díaz & Cabido 2001). When traits are categorical, species can be aggregated into functional groups (or types) that share the same functional attribute (e.g. xerophytic species versus mesophytic species), and their number or kinds are a measure of functional diversity (Díaz& Cabido 2001).

Selecting the right organisms to use as ecological indicators, is as important as selecting the most responsive biodiversity metrics to climate change. Ecological indicators of atmospheric changes, such as climate change, must meet several criteria to expedite its interpretability and application (Pinho, Máguas & Branquinho 2010): (i) as dependent on the atmosphere as possible, lacking influences from other environmental sources such as soil and its water content; (ii) easy to measure; (i) respond

to the environmental stress in a predictable and comprehensive way; (iii) producing a robust indicator with low variability; (iv) integrate both spatial and temporal changes; (v) inter-regionally compatible; (vi) whenever necessary, exhibit thresholds to help determine management actions. Most vascular plants lack these requirements, being, for example, affected by soil properties and reflecting their nutrient and water contents. Most vascular plants lack these requirements, being, for example, affected by soil properties and reflecting their nutrient and water contents. On the other hand, epiphytes grow nonparasitically on plants and rely almost entirely on the atmosphere for nutrient or water supply. However, not all epiphytes are suitable. Some, like bromeliads, orchids or ferns, do not directly reflect atmospheric water and nutrient content, because they have structures such as cuticle or stomata that enable them to regulate their content. Poikilohydric organisms, such as lichens and bryophytes lack those structures. They are ideal ecological indicators of atmospheric change, as they respond directly to the levels of water and nutrients present in the atmosphere. Lichens show several advantages over bryophytes as ecological indicators. They exist in every terrestrial ecosystems, including the driest from which bryophytes are almost excluded. They are available for collection all year round and are comparably slow growing organisms, without seasonal variations in morphology, integrating perfectly spatial and temporal patterns of atmospheric changes. This is the reason why they have been used for more than 100 years as ecological indicators environmental change.

lichens as ecological indicators

Lichens are a symbiotic association between a fungus and a photosynthetic partner (green algae and/or cyanobacteria, (Hawksworth & Honegger 1994). They are excellent ecological indicators because they are rootless poikilohydric organisms, whose water and nutrient levels are mostly regulated by atmospheric conditions, to which they directly respond. Their inability to regulate water content, which fluctuates with the surrounding environment (Green, Sancho & Pintado 2011), ends up regulating their physiological activity, and under humid conditions they become wet and activate, while under dry conditions they dehydrate and became inactive. They are also poikilothermic, so they respond as well to temperature fluctuations in the atmosphere and these regulate also their physiological activity. These physiological features, allied to their wide distribution along almost all terrestrial ecosystems (from the cold Poles to the hot deserts), make lichens highly sensitive and responsive to a wide range of anthropogenic and natural disturbances, including climate, being capable of depicting its spatial and temporal variation.

Since the beginning of the industrial revolution to present days, lichens have efficiently tracked the major drivers of global change. They are amongst the most sensitive organisms to environmental change, signaling it before other less sensitive components of the ecosystems (Pinho *et al.* 2009; Pinho *et al.* 2011; Pinho *et al.* 2014) and this sensitivity to atmospheric change is the reason why they have been used for more than a century in biomonitoring studies. More than a decade ago, a global meta-analysis by (Parmesan & Yohe 2003) revealed climate change fingerprint of temporal and spatial species range shifts and lichens were within the vast set of taxa included. The meta-analysis referred to a study where lichen compositional shifts were observed within a 5 years interval in response to global warming (van Herk, Aptroot & van Dobben 2002). These results were later confirmed by another work in the same area, leading the authors to suggest that lichens could be among the most responsive organisms to climate change (Aptroot & van Herk 2007).

standard methods to sample lichen diversity

Nylander was the first in the 19th century to recognize that lichen absence in the Jardins du Luxembourg was due to air pollution (Gilbert 1973). It would take a century before the first scale was developed to estimate SO_2 pollution deposition, based on the presence of certain lichen species (Hawksworth & Rose 1970). Since then, several methodologies have been tested to assess lichen diversity. The first widely used method to assess total lichen diversity was called the Index of Atmospheric Purity (IAP; LeBlanc & Sloover 1970). The method was broadly accepted in Europe, particularly in Italy and Germany, (Conti & Cecchetti 2001) and its repeated use in these countries lead to the standardization of its procedure and the establishment of guidelines (VDI 1995). Following these developments and also the approach suggested by a Swiss team (Ammann *et al.* 1987), a modified and improved version of the IAP was developed to assess lichen diversity values (LDV, Asta *et al.* 2002). At the same time, in the US another methodology was developed (USDA 2011) to assess lichen diversity as part of the US Forest Service Forest Health Monitoring Program, under the auspices of the US Environmental Protection Agency, designed to monitor air quality and climate change in all US territory over time. Additional methods have been developed in other regions, like for instance the Brazilian rubber band method (Marcelli 1992), but its application has been restricted to small-scale local projects.

During this process to develop sampling methodologies, lichen diversity metrics have also evolved. In general, methodologies started to account only with species richness (Gilbert 1973). This was sufficient until the 1980s, when SO_2 dioxide was the major driver of change. In cases where the pollutant has an

overall deleterious effect on most species, like SO_2 or copper (Branquinho *et al.* 1999), species richness directly correlates with the environmental driver. However, some pollutants may primarily induce a shift in species composition, and, in moderate levels, may even increase the number of tolerant species, like in the case of nitrogen pollution (van Herk, Aptroot & van Dobben 2002; Pinho *et al.* 2011). Thus, methodologies started to include measures of species frequency or abundance. Simultaneously, scientists realized the need to account with phorophyte bark texture, bark chemistry, and canopy drip particularly in grid-based sampling methods (Conti & Cecchetti 2001; Llop *et al.* 2012). These methodologies soon adopted a list of unsuitable phorophytes (with a peeling bark, for instance), and suitable and exchangeable phorophytes to facilitate their use across broad geographical scales. Currently, two standard methodologies to sample lichen epiphytic diversity are routinely used at the continental level: the EU and the US standard methodologies.

The European standard methodology is the methodology developed and improved by Asta and co-workers (2002). Following some enhancements to standardize its operation procedures and harmonize the consistency of the assessments, at national (Giordani *et al.* 2009; Brunialti *et al.* 2012) and European levels (Cristofolini *et al.* 2014), the method was recently adopted under the Comité Européen de Normalization (CEN) framework (Ambient air – Biomonitoring with lichens – Assessing epiphytic lichen diversity. European Standard EN 16413:2014). The method samples all lichen species (macro and microlichens) occurring inside a size-standardized grid, placed on the four cardinal directions of a tree trunk (the number of trees per plot and the size of the plot are decided upon according to the environmental problem being studied). The resulting metrics are species richness and their frequency. This method provides a reliable, consistent and objective standard procedure to assess lichen diversity, ensuring data quality and comparability in space and time and it is used across all Europe (Cristofolini *et al.* 2014).

The second method was developed by the US Forest Service (USDA 2011) to monitor air quality and climate change and has been used for more than 20 years. This method underwent also testing for repeatability and has its own quality assurance and control features (McCune *et al.* 1997), including annual certification of surveyors. Unlike the European method, surveyors search across a large circular sampling plot (~4200 m^2) for a maximum of two hours and don't need to be trained lichenologists. All macrolichens species detected on any tree or shrub > 1 m above ground inside the plot are visually rated for their abundance on a 1 to 4 exponential scale and vouchered for later identification by a trained lichenologist. The lichen metrics

obtained are species richness and abundance. The method is routinely used in the US and also in some parts of Canada and Mexico due to its reliability and compatibility of the data obtained across space and time.

In the current context of global change, it is imperative that both the impact of global change drivers, such as climate change, and the global effect of political measures (the Paris Agreement, for example) adopted in response are evaluated. This can only be done if standardized methodologies are applied at worldwide spatial and long-term temporal scales. Moreover, a global biodiversity monitoring network is set in motion to assess and keep track of the effects of global change (Pereira & Cooper 2006; Scholes *et al.* 2012). However, for retrospective and future analysis, the inclusion in this monitoring network demands that data collection is based on compatible standardized methodologies performed at regional and global scales (Mace *et al.* 2005, MEA, 2005, Pereira & Cooper, 2006). The EU and US methods are used at a continental scale (North America and Europe) and could be used for global trend analysis at these continental scales. Thus far, cross-continental trends still lack, because there is no widely accepted universal methodology to assess lichen diversity at a global scale, and because the existent methods compatibility was never investigated.

lichen functional diversity

Standardization of sampling procedures is as fundamental for the generalization of relationships between global change drivers and effects, as are the metrics used to analyse biodiversity response at a global scale. As previously mentioned, functional diversity assessments can provide a crucial tool to do this. Promising results have been achieved using the functional characteristics of plants as ecological indicators (Lavorel 2013; Spasojevic *et al.* 2013). However, the use of lichen traits, as an ecological indicator of global change is far less developed (Marini, Nascimbene & Nimis 2011; Pinho *et al.* 2012; Giordani *et al.* 2014). A few works used epiphytic lichen functional traits as an indicator of micro or macroclimate (Giordani & Incerti 2008; Pinho, Máguas & Branquinho 2010; Marini, Nascimbene & Nimis 2011; Giordani *et al.* 2012; Giordani *et al.* 2013; Colesie *et al.* 2014; Concostrina-Zubiri *et al.* 2014; Root *et al.* 2015). Yet, works specifically aiming to identify lichen functional traits responding to climate remain poorly explored and its application as an ecological indicator of climate change is still lacking.

Unlike for plants (Kattge *et al.* 2011), there is no international database covering lichen traits. Most works on Southern Europe based their functional groups classification on the Italian database (Nimis & Martellos 2008), which classifies species

fig. 3. Examples of lichen traits. Main types of photobiont: (a) *Trentepohlia*; (b) other green algae; (c) cyanolichens; (d) jelly cyanolichens.

into response traits (e.g. eutrophication tolerance or light preferences) according to expert knowledge based on ecological performance. Although good results were achieved for nitrogen lichen responses (Pinho *et al.* 2011), as it is a national database, its application may be more difficult for works outside Italy. A good first approach to make trait analysis independent of expert judgment and geographical area would be to start with traits easy to identify. Lichens main type of photobiont and growth form (Fig. 3 and 4) are easily measurable lichen response traits that do not need identification to the species level, an important feature to consider when looking for universal ecological indicators.

The photobiont component is particularly responsive to water availability and source (Gauslaa 2014). Three main types of photobiont may be found, and these constitute three different functional groups within this trait. Cyanolichens need liquid water to initiate photosynthesis (Lange, Kilian & Ziegler 1986; Green, Sancho & Pintado 2011), and are therefore more frequent in areas with higher precipitation (Fig 3 C and D) or with high dew formation (Gauslaa, 2014). On the other hand, green algae lichens are able to start photosynthesis using water vapour alone (Fig. 3 A and B; Green *et al.* 2011), so they are able to explore a wider range of water sources and are geographically more widespread because they are not so strongly limited by dew and

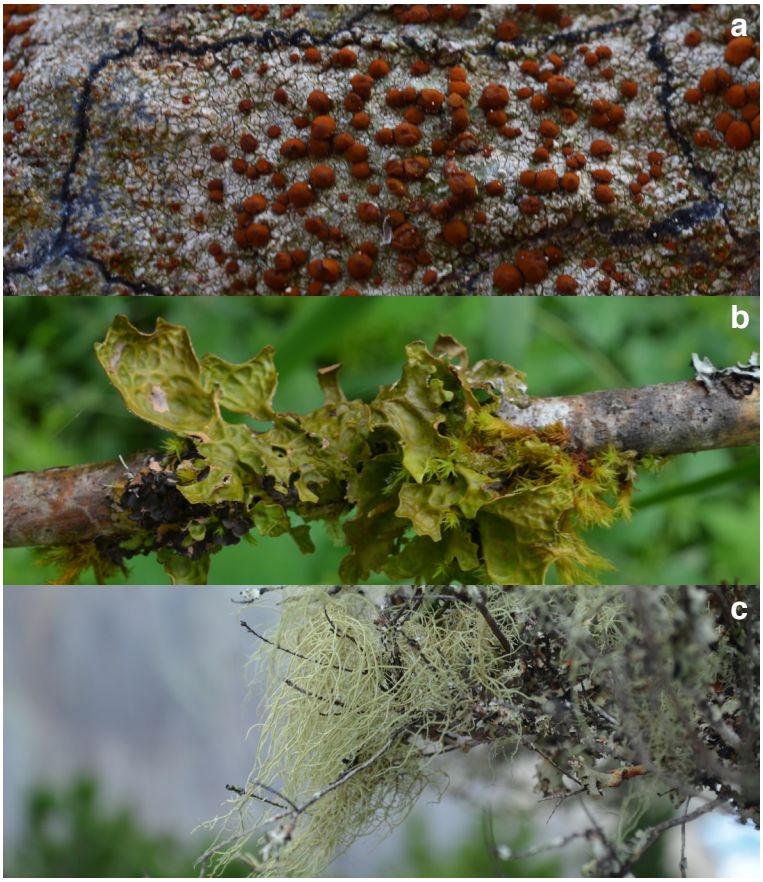

fig. 4. Examples of lichen trait. Growth forms: (a) crustose; (b) foliose; (c) fruticose.

precipitation patterns. Green algae lichens with *Trentepohlia* algae are usually considered separately (Fig. 3 A). This algae has a narrower range of optimal temperatures and is usually associated to high temperatures and moisture, being frequenter in sub-tropical and tropical conditions (Rundel 1978; Nimis & Tretiach 1995; Rindi & Lopez-Bautista 2008). Lichens growth form is also related to the way they absorb water and particles from the atmosphere. For this reason, each of the different growth form types can be considered as a functional group within this trait. Fruticose species with their shrub–like form are well adapted to explore air moisture or dew (Fig. 4 C), foliose (leaf-like) species require more liquid water (Fig. 4 B) (Gauslaa 2014) and crustose species are generally less limited and can live under a wider range of conditions (Fig. 4 A). Other lichen traits might be related to climate, like their ability to synthesize numerous chemical compounds (Concostrina-Zubiri *et al.* 2014) or the ratios of photobiont/fungal biomass (Gauslaa & Coxson 2011). However, these traits are harder to determine and were not considered in this work.

traits response to climate

We know from functional diversity theory that traits or functional groups are better to relate with environmental variables since the environment works as a filter for traits (Suding *et al.* 2008; Villéger *et al.* 2010; Mouillot *et al.* 2012; de Bello *et al.* 2013). Thus, traits relation with the environment (or functional groups relation) is expected to be more consistently monotonic so potentially more predictable than that based on other biodiversity metrics such as species richness (Mouillot *et al.* 2012).This is essential if we want to develop trait-based ecological indicators capable to predict climate change effects on the ecosystems. Nonetheless, before we can incorporate trait-based ecological indicators for better predictions in a context of climate change, we need to know what are the essential climate variables driving lichen traits response. This is necessary to better comprehend lichen community responses and to correctly interpret the ecological indicators. Because functional traits enlighten us on how environmental factors shape biodiversity (de Bello *et al.* 2013), this will be pivotal to show us which are the essential climate variables driving changes in biodiversity patterns, and thus those that should be kept under observation in the future.

space for time substitution

Understanding ecological responses at multiple spatial and temporal scales is difficult without long-term observations. To overcome this limitation, ecological studies have long relied on space-for-time substitutions, a technique based on the assumption that spatial and temporal sequences are similar (Delcourt & Delcourt 1991). This approach has enabled researchers to understand and model future or past temporal processes, that would have been otherwise difficult to observe (Blois *et al.* 2013). The development of ecological indicators of climate change based on lichen traits relies also on the recognition of generalised relationships between climate drivers and lichen traits responses at multiple spatial and temporal scales. However, we lack enough long-term observations to do this and we cannot afford to wait until we have enough information. Space-for-time substitutions play here a crucial role. If we are able to identify and develop ecological indicators capable of signalling specific climate shifts along space, that will enable us to apply them to signal such shifts in climate over time. However, we still need long-term observations to understand the extent to which inferences from spatial analysis can in fact uphold over time (Fukami & Wardle 2005). This is essential to validate the developed ecological indicators efficiency to track climate change over time and to understand short-term and long-term responses, for better predictions of the effects of climate change (Dunne *et al.* 2004).

aim & rationale

The general objective of this thesis is to develop the use of lichen functional diversity as an integrated and global ecological indicator of climate change (Fig. 5). **Chapter one** provides a general introduction framing the problematic of global change drivers with particular emphasis on climate change and the need for ecological indicators capable of tracking its effects on ecosystems. Due to the global nature of climate change, it is imperative that both the ecological indicators and the metrics are universal so they can be globally applicable.

To build universal ecological indicators it is crucial that standardized compatible methodologies are applied at broad spatial and temporal scales. Though no widely accepted method is available to sample lichen biodiversity, two methods are currently used at the continental level: North America and Europe. It would likely be a Herculean task to convince scientists on both continents to adopt one of the methodologies, or to develop a new common one. If achieved, comparing trends in retrospective data would still be a problem. In **chapter two** our solution is presented. We develop a framework to compare data generated by these two lichen survey protocols in terms of the most widely used biodiversity metrics to track global change, featuring paired datasets from the northwestern US in response to two major drivers of environmental change (nitrogen deposition and climate). We expect that despite their basic differences in the sampling process, the main outputs can be interpreted in the same way.

Standardization of sampling procedures is as fundamental for the generalization of relationships between global change drivers and effects, as are quantitative methods to analyse biodiversity response at a global scale. Functional diversity assessments can provide a crucial tool to assess the effects of drivers of global change, accounting with species redundancy and with the potential to be both universal and applicable at large spatial scales, due to its independency from species identity. Nonetheless, lichen functional diversity in response to climate remains poorly explored. In **chapter three** we give the first steps in the identification of easy measurable lichen functional traits responding to an aridity gradient featuring a dataset from southwestern Europe. This first community level approach is focused on traits that require minimum expert knowledge, with the intention to boost its global application. Aridity was chosen as the climate variable to start with because it integrates several basic climate variables (precipitation, temperature and potential evapotranspiration). In **chapter four** we detail this response, using all the available basic climate variables (precipitation, temperature and relative humidity) and each of the lichens functional groups (belonging to the previously identified traits).

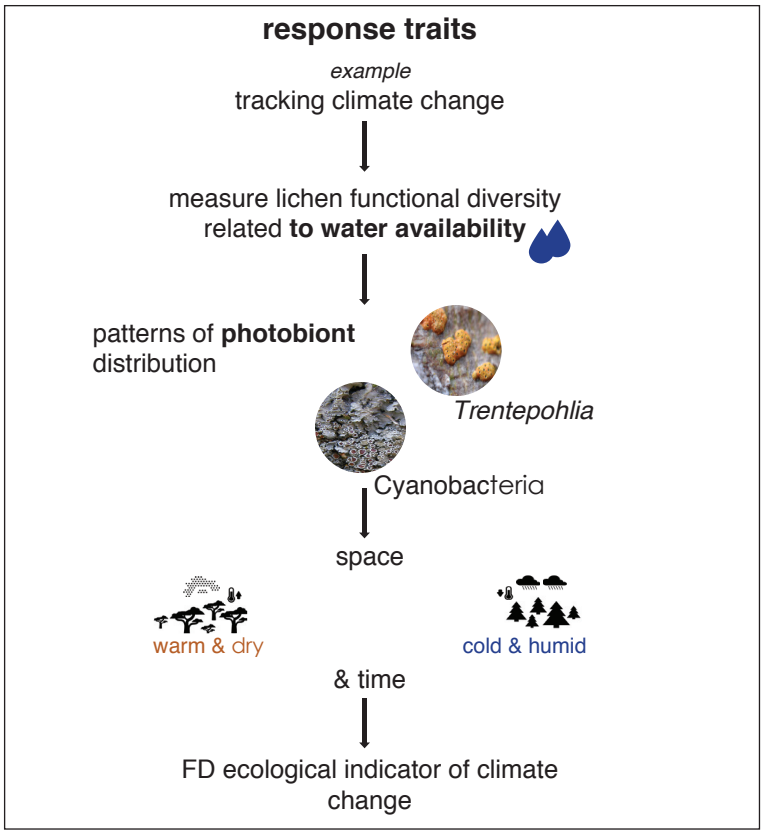

fig. 5. Framework of the thesis.

This will help us recognize the specific climate variables driving lichen traits and functional groups response, helping us understand which climate variables should be tracked in the future.

In **chapter five**, we explore lichen traits relation with climate to understand if it is predictable and usable as an ecological indicator of climate change, and develop ecological indicators of climate change based on lichen functional groups. Because the trait-based approach is not limited by species identity, this chapter explores also its global application, featuring a dataset from southern Europe along several climates. The successful application of these ecological indicators of climate change developed using a space-for-time proxy relies on its validation over time. In **chapter six** we validate this approach with a dataset from southern Portugal collected over fifteen years at a five years interval (1994 to 2010).

Finally, in **chapter seven**, we discuss the work developed. This chapter aims to open new perspectives on lichen functional diversity potential as a universal indicator, showing the progresses made thus far and future challenges. How we started from scratch, building a universal sampling framework. We discuss lichen traits response to climate, namely the predictability of its responses and its potential global application, with an example

using datasets from several climatic regions in the world, namely USA, Brazil, Portugal, Spain, Italy and Thailand. Afterwards we discuss how should the ecological indicators be applied and how space-for-time substitutions can be used to predict climate change effects based on these ecological indicators. In this chapter, knowledge gathered over the previous chapters is integrated. Our aim is to understand if using a standard sampling methodology, a functional group approach and a space-for-time proxy we are able to get closer to a global integrated ecological indicator of climate change.

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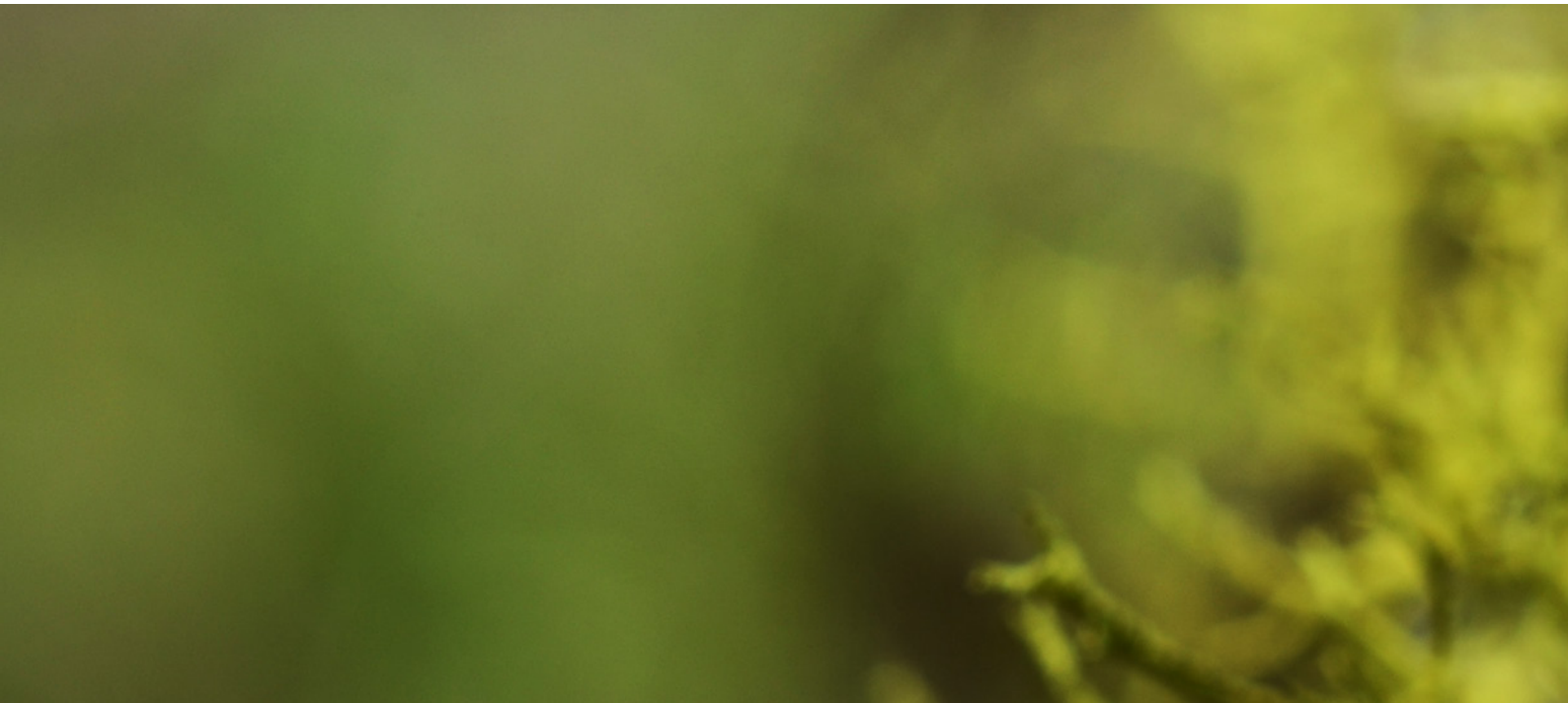

chapter two

Tracking global change using lichen diversity: towards a universal framework

publication

Matos P., Geiser L., Hardman A., Glavich D., Pinho P., Nunes A., Soares A.M.V.M. & Branquinho C. Under review in Methods in Ecology and Evolution.

abstract

Lichens have been used to efficiently track the major drivers of global change at the local to regional scale, from the beginning of the industrial revolution (sulphur dioxide) to the present (nitrogen deposition, and climate change). Currently, the challenge is to universalise monitoring methodologies to compare global change drivers' effects and to assess the efficacy of mitigation measures at various spatial and temporal scales.

Because two protocols are now used at a continental scale (North America and Europe), it is timely to investigate the compatibility of their respective results. Our aim is to stimulate interest in lichens as global scale ecological indicators and facilitate analyses of existing lichen datasets to track the effects of major global change drivers. We present an analytical framework to compare these datasets using broadly accepted biodiversity metrics.

For the first time, we made direct comparisons of results between lichen survey data from EU and US methodologies featuring a paired dataset from the US Pacific Northwest. Methodologies yielded highly similar trends in taxonomic diversity, functional diversity, and community structure in response to two major drivers of global change (nitrogen deposition and climate). A framework was designed to incorporate measures of species richness (to measure biodiversity loss), shifts in species composition (to measure compositional turnover) and measures of functional diversity (to provide a link between community shifts to effects and ecosystem structure and functioning) allowing a joint analysis of US and EU datasets. Incorporating these biodiversity metrics, which are the most currently accepted, is essential to more thoroughly comprehend biodiversity response to global change. This framework enables future cross-continental analysis of lichen biodiversity trends from North America and Europe in response to global change, taking us one step closer to a lichen-based universal ecological indicator. This is essential to provide the institutions responsible for creating and implementing environmental legislation the information needed to make and monitor policies and adaption measures.

keywords

Ecological indicators, nitrogen deposition, climate change, functional diversity, community shifts, taxonomic diversity, Europe, North America

introduction

In the present context of global change, the scientific community is challenged to develop tools to compare and assess the impacts of global change drivers, and the effects of the political measures adopted in its response (Branquinho, Matos & Pinho 2015). In this regard, the United Nations Conventions on Biological Diversity (UNCBD), Climate Change (UNCCC) and

to Combat Desertification (UNCCD), have long demanded a set of globally applicable ecological indicators. Monitoring these ecological indicators is fundamental to track the impacts of global change drivers, to measure progress towards the targets set by these conventions, and to improve and guide new strategies for biodiversity conservation (Pereira & Cooper 2006). The framework for attaining this global level of available and integrated biodiversity-related data is set in motion (Scholes *et al.* 2012). However, biodiversity indicators are far from being completely developed, and are highly biased towards population indicators based on vertebrates (Pereira, Navarro & Martins 2012). Data from less known taxonomic groups are needed in this global monitoring network, but for its inclusion is essential that data collection is based on compatible standardized methodologies performed at regional and global scales (Mace *et al.* 2005; MEA 2005; Pereira & Cooper 2006).

Epiphytic lichen diversity has been used to efficiently monitor the major drivers of global change from the beginning of the industrial revolution (sulphur dioxide; Hawksworth & Rose 1970; Gilbert 1973) to the present (nitrogen deposition, and climate change; (Davies *et al.* 2007; Branquinho, Matos & Pinho 2015; Matos *et al.* 2015). Modern lichen-based environmental analyses provide low cost, high-resolution spatial tools for modelling and mapping environmental change, allowing its detection, assessment and monitoring (Branquinho *et al.* 2008; Pinho *et al.* 2008). They are powerful ecological indicators of pollution (Giordani, Brunialti & Alleteo 2002; McMurray, Roberts & Geiser 2015) and macro and microclimate (Aptroot & van Herk 2007; Pinho, Máguas & Branquinho 2010; Matos *et al.* 2015), and provide also a valuable tool for forest, natural resource, and biodiversity assessment and conservation (Nascimbene, Marini & Nimis 2007; McMurray, Roberts & Geiser 2015). Lichen-based thresholds were used to establish the lowest critical levels for nitrogen concentration and critical loads for nitrogen deposition in Europe and the USA, contributing to the protection of ecosystem services and functions in both natural and semi-natural ecosystems (Cape *et al.* 2009; Pardo *et al.* 2011; Pinho *et al.* 2012; Root *et al.* 2015). Thus, their established value as ecological indicators can also aid decision-making in management and regulatory arenas (Blett *et al.* 2014).

Since the 1970s, many different methods were used to assess lichen diversity, impairing comparisons of the resulting data at high spatial scales (cross-continental or global level). Among these, two standardized methodologies for sampling lichen epiphytic diversity predominate presently, one in Europe (EU) and another in the US. The European standard methodology was developed by Asta and co-workers (Asta *et al.* 2002) and was recently adopted under the Comité Européen de Normalization

(CEN) framework (Ambient air – Biomonitoring with lichens – Assessing epiphytic lichen diversity. European Standard EN 16413:2014). The US method was developed by the US Forest Service (USDA 2011) to monitor air quality and climate change, and it is also used in Canada and Mexico. The methods differ largely in two main aspects: 1) the EU method records all lichen species (macro and microlichens) occurring inside a size-standardized grid placed on a tree trunk in a fixed number of trees, resulting in metrics of frequency; 2) the US method surveys all macrolichen species detected on any tree or shrub inside a large circular sampling plot (0.4 ha), visually rating species abundance. Although desirable, it would likely be an Herculean task to convince scientists on both continents to adopt only one of the methodologies, or even to develop a new common one. If achieved, we would still have problems using retrospective data. The solution could be to investigate these methods compatibility and develop a way to use them under a trend analysis approach. This would enable cross-continental analysis of lichen monitoring data under a global change perspective, and possibly allow its inclusion in the global monitoring network.

Assessing methods compatibility under a global change perspective requires a proper selection of biodiversity metrics. Candidates for a set of essential biodiversity variables have been proposed as the basis for these monitoring programs worldwide, in an effort to measure the multidimensional nature of biodiversity change (Pereira *et al.* 2013). Species richness metrics are necessary because they measure the irreversible component of biodiversity (species extinctions, biodiversity loss), at local, regional or global scales (MEA 2005; Pereira *et al.* 2010; Pereira, Navarro & Martins 2012). However, biodiversity loss metrics cannot be so immediately linked to ecosystem services and are not so responsive to global change drivers (Balmford, Green & Jenkins 2003; Dornelas *et al.* 2014). Rather than decreased diversity, global change drivers trigger a shift in species composition and abundance (Balmford, Green & Jenkins 2003; Sax & Gaines 2003; Dornelas *et al.* 2014). Hence, shifts in community structure, integrating measures of species abundances, should be considered as they are more responsive (Balmford, Green & Jenkins 2003; Dornelas *et al.* 2014). Because functional diversity is a more universal indicator of community changes (independent of species identity) and is linked to ecosystem functionality in response to anthropogenic drivers across broad spatial scales and environmental gradients (Lavorel *et al.* 2011), measures of functional diversity should likewise be considered.

Here, we present a framework to jointly analyse data generated by the two most widely used lichen survey methods using broadly

accepted biodiversity metrics to track the effects of global change. We aim to answer these questions: Can lichen data acquired by two different survey methodologies, US and EU, give comparable results along climate and pollution gradients? If so, how should they be compared under a global change perspective? To do this we determined lichen epiphyte diversity using both methods at 28 sites spanning regional nitrogen deposition and climatic gradients in the northwestern US. Climate and nitrogen pollution gradients were included because these are currently some of the emergent and most pressing drivers of global change (Steffen *et al.* 2015). Methods compatibility was assessed comparing their performances in terms of trends in taxonomic diversity (species richness, Shannon, Simpson and evenness diversity indices), shifts in species composition and functional diversity (community weighted mean, CWM) along these gradients.

materials & methods

STUDY AREA AND SAMPLING SITES

The study area was located in the northwestern United States (Fig. 1). Eleven low elevation sites were surveyed in the Columbia River Gorge National Scenic Area following a pollution gradient from Portland, Oregon. The remaining sites were on-frame USFS Forest Inventory Analysis P2 plots (http://www.fia.fs.fed.us/library/field-guides-methods-proc/index.php) in the temperate rain forests of the western Oregon and Washington Cascades [Rogue River-Siskiyou (1), Mount Hood (1), and Gifford-Pinchot (2) national forests] and dry coniferous national forests of the eastern Cascades and Blue Mountains of Oregon [Fremont-Winema (6), Ochoco (1), Umatilla (5), and Wallowa-Whitman (1)]. Sites were visited during June and July 2013. Elevational, climatic and pollution profiles are given for each of these areas in Table 1.

US LICHEN SAMPLING METHOD

The US method to survey epiphyte macrolichens followed the Forest Inventory and Analysis (FIA) lichen community indicator protocol (USDA 2011). Briefly, a trained surveyor circumambulates a circular plot of 0.38 ha for up to 2 h, collecting a voucher of each epiphytic macrolichen species detected on woody plants above 0.5 m from the ground: including trees boles, branches and twigs, and branch litter, saplings and shrubs, and standing dead trees. For each species detected, an abundance rating is assigned based on the number of individual thalli observed during the survey: 1 - rare (1-3 individuals); 2 - uncommon (4-10 individuals); 3 - common (>10 individuals, occurring on less than half of available substrates); 4 - abundant (species present on more than half of all substrates). So, a species that was detected

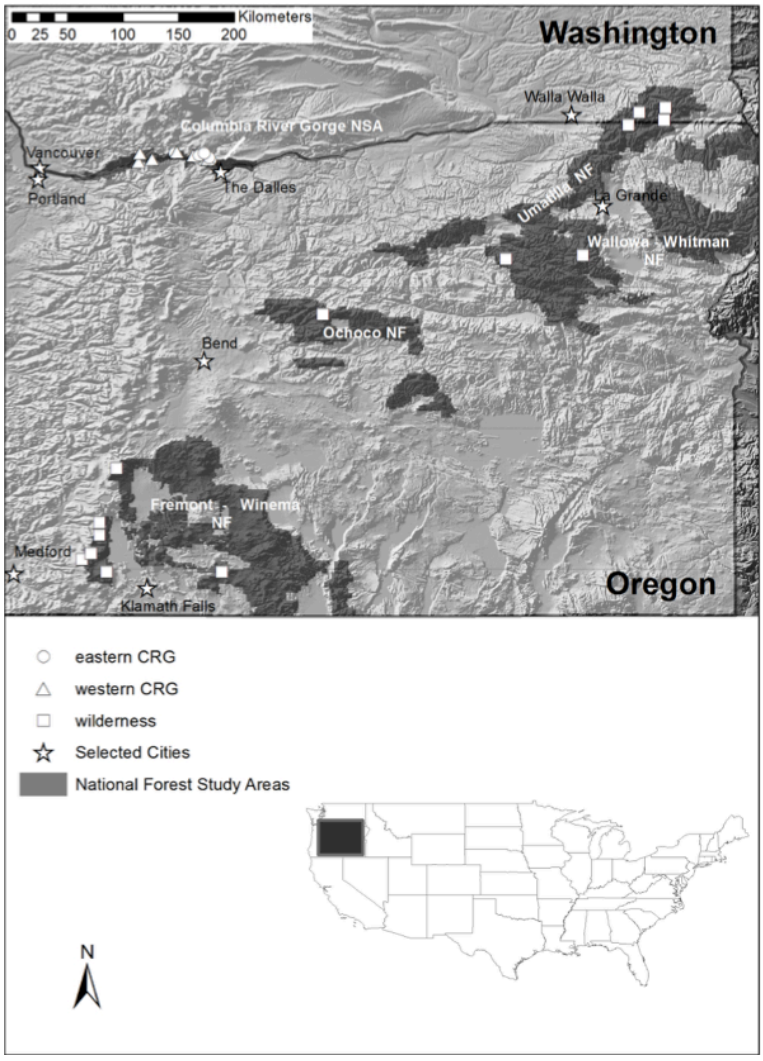

fig. 1. Map of the study area showing sampling plots location, in northwestern US. Different symbols represent different geographic and climatic areas, as grouped by cluster analysis. N = 28. Squares represent plots in high elevation Wilderness Areas (14), circles plots in eastern Columbia River Gorge (8), and triangles plots in western Columbia River Gorge (6).

on more than half of the trees received an abundance rating of 4. All vouchers were identified in the laboratory to species following McCune & Geiser (2009) and Esslinger (2012).

EU LICHEN SAMPLING METHOD

To apply the method to the US plots, a minimum of 4 and maximum of 8 trees near the centre of the plot were selected for sampling, determined by the time spent on each tree and the total time (2 to 3 hours) available to survey the site. At 130 cm above ground level, each tree selected: i) had a trunk circumference of 50-250 cm; ii) was not leaning more than 20º; and iii) had a clear area on the trunk without damage, decortication, branching, knots, or other epiphytes preventing lichen growth. A 10 x 50 cm frame divided into five 10 x 10 cm grid cells was placed on the north face of the tree trunk. The uppermost edge of the frame was positioned at 150 cm from the ground level, adjusted up to a maximum of 2 meters height if the trunk was unsuitable at

table 1. Range of elevational, climatic and pollution conditions of the study sites, as grouped by cluster analysis: E CRG - Eastern Coumbia River Gorge; W CRG - western Columbia River Gorge; HWA High elevation Wilderness areas. N = 28. Climate variables: E - elevation, Tmax - maximum temperature in August, Tmin - minimum temperature in December, T - annual mean temperature, P - annual precipitation and RH - relative humidity. Pollution variable: N - nitrogen deposition given in terms of dry weight N concentrations in lichens.

	E (m)	Tmax (ºC)	Tmin (ºC)	T (ºC)	P (cm)	RH (%)	N (lichen N kg/ha/yr)
E CRG	58 - 462	28.3 - 29.8	-3.4 - -1.8	9.6 - 11.1	46.1 - 63.8	45 - 52	1.61 - 6.42
W CRG	66 - 617	26.0 - 27.8	-2.7 - -0.1	9.6 - 10.9	70.7 - 191.8	52 - 55	1.01 - 2.11
HWA	1298 - 2267	20.4 - 27.6	-10.3 - -4.0	3.9 - 8.0	42.7 - 143.1	38 - 51	0.58 - 2.47

the desired height (e.g. to avoid snow lines or branches). Each lichen species occurring inside each grid cell was identified and recorded or was collected for later laboratory identification. Sampling was repeated on the S, E, and W-facing sides of the trunk. Lichen abundance (frequency) was recorded as the number of grid cells (out of 20 possible) in which each species was detected. Lichen identification followed McCune & Geiser (2009), McCune (2012) and Esslinger (2012). The LDV (Lichen Diversity Value) index was calculated for all species (EU) and for macrolichens only (EUm) following Asta *et al.* (2002). The frequency for each species (species LDV) is calculated as the mean frequency on all the trees sampled at the site.

LICHEN FUNCTIONAL DIVERSITY

Each macrolichen species was assigned to a N-sensitivity functional group (**Supplementary Table S1**) related to the depositional loading (kg N/ha/yr) above which the probability of detecting the species declines in the US northwest: oligophilic (< 2.6); mesophilic (2.7 - 8); nitrophilic (> 8). Ratings follow Geiser *et al.* (2010) and Root *et al.* (2015) for species west and east of the Cascades Range crest, respectively. When species scored differently east and west of the Cascades Range crest, east score was given (due to the highest number of sites in this part of the range). Because these previous studies did not contemplate microlichens, only macrolichens could be assigned to functional groups.

Species functional group assignments were combined with species abundance data from each sampling method (US, EU, and EUm) to obtain the community weighted mean (CWM; Lavorel *et al.* 2008) for each functional group at each plot. This index represents the mean functional group value in the community, weighted by the abundance of species belonging to that functional group (Lavorel *et al.* 2008). This was calculated using the 'dbFD' function of the CRAN software R (R Core Team 2013), FD package (Laliberté & Legendre 2010).

CLIMATE AND POLLUTION DATA

Estimates of thirty years normal (1970-2000) annual mean precipitation, temperature, and relative humidity were extracted from the Parameter-elevation Regressions on Individual Slopes Model (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 15 August 2013) at an 800 m cell resolution using a GIS overlay on the plot coordinates. Twenty grams of *Letharia vulpina* or *Platismatia glauca* were collected and analysed for total elemental N following Geiser (2004). Dry weight N concentrations (%) in lichen thalli were used to estimate canopy through-fall deposition of total N from nitrate and ammonium ions following Root *et al.* (2013).

DATA ANALYSIS

Four taxonomic diversity indices (species richness, Shannon-Wiener index, Simpson's index and Pielou's evenness) were computed per plot using the species abundance matrices for all species (EU) and macrolichens only (US and EUm). We calculated Pearson correlations (r) between indices derived from the different sampling methods (correlations were considered significant for $p < 0.05$).

Plots were aggregated into distinct geographical and/or climatic areas using hierarchical, agglomerative cluster analysis with Euclidean distances and Ward's linkage method, based on the three species matrices derived from the US and EU methods using PC-Ord Software Version 6.08. The choice of optimum number of groups to prune the dendrogram was done using Indicator Species Analysis (ISA; Dufrêne & Legendre 1997). Plots were clustered into up to 17 groups and an ISA analysis was performed on the climate matrix for each group memberships. Resulting *p*-values for each climate variable were averaged for each level of grouping and the number of significant ($p < 0.05$) indicator climate variables was registered. The optimal number of groups was chosen pondering the lowest *p*-value with the highest number of indicator climate variables (McCune, Grace & Urban 2002). Significance of the groups formed was assessed using multi-response permutation procedures (MRPP), with groups considered significantly different if $p < 0.05$.

Non-metric multidimensional scaling (NMS) was used in the EU, US, and EUm species matrices to extract prominent gradients in lichen community composition. When sampling sites include a large gradient with very distinct local site characteristics unrelated to the environmental gradient of interest, values from the EU method are usually relativized to prevent biasing results

and impairment of comparisons (Matos *et al.* 2015). Because the US species abundance scale is log-like, values from EU species matrices were log transformed after relativizing. Though this transformation improves comparison outcomes (similarity between distance matrices and ordination scores improves around 3%), this is not an essential step. The best NMS solution was run with Bray-Curtis distance (McCune, Grace & Urban 2002), chosen from 500 runs, each starting randomly (500 iterations per run), and evaluated with a Monte Carlo test (250 runs with randomized data). The coefficients of determination (r^2) between the original plot distances and distances in the final ordination solution were calculated to assess how much of the lichen community variability was represented by the NMDS axes (McCune, Grace & Urban 2002). Climate, pollution and functional variables were overlaid on the NMS ordination as correlation vectors (McCune, Grace & Urban 2002). Individual correlations between these variables and NMS site scores were determined using Pearson correlations (correlations were considered significant for $p < 0.05$). Correlation (r) and redundancy (%) between ordinations were assessed using a Mantel test (ordinations were considered related for $p < 0.05$). Associations between distance matrices obtained with both methods were assessed using the Mantel test (matrices were considered associated when $p < 0.05$).

results

Species richness and Shannon-Wiener indices correlated strongly between US and EU (total and only macro) datasets (Fig. 2). This was also observed for Simpson index ($r = 0.78$, $r = 0.80$ and $p < 0.001$, respectively). Species richness was higher in the EU dataset, and lower in the EU macrolichen only dataset. Conversely, the US method yielded higher values of Shannon-Wiener and Simpson's indices than both EU or EUm datasets. Pielou's evenness index values for EU vs. US datasets were weakly correlated ($r = 0.38$, $p = 0.044$); and we found no significant correlation between macrolichen only datasets (EUm vs. US; $r = 0.25$, $p = 0.199$).

A cluster analysis of the US and EU species matrices assigned the same sampling sites to the same three groups when the dendrogram cut-off was set to three groups. These groups represented three biogeographical and climatic areas: high elevation Wilderness Areas, the eastern Columbia River Gorge, and the western Columbia River Gorge (Fig. 1).

NMS extracted nearly identical environmental gradients from data generated by both methodologies. The ordination (Fig. 3) shows the NMS solution for the location of the sampling sites in species space, i.e. based on lichen community composition. The analysis suggested a two-dimensional solution for all three datasets; the

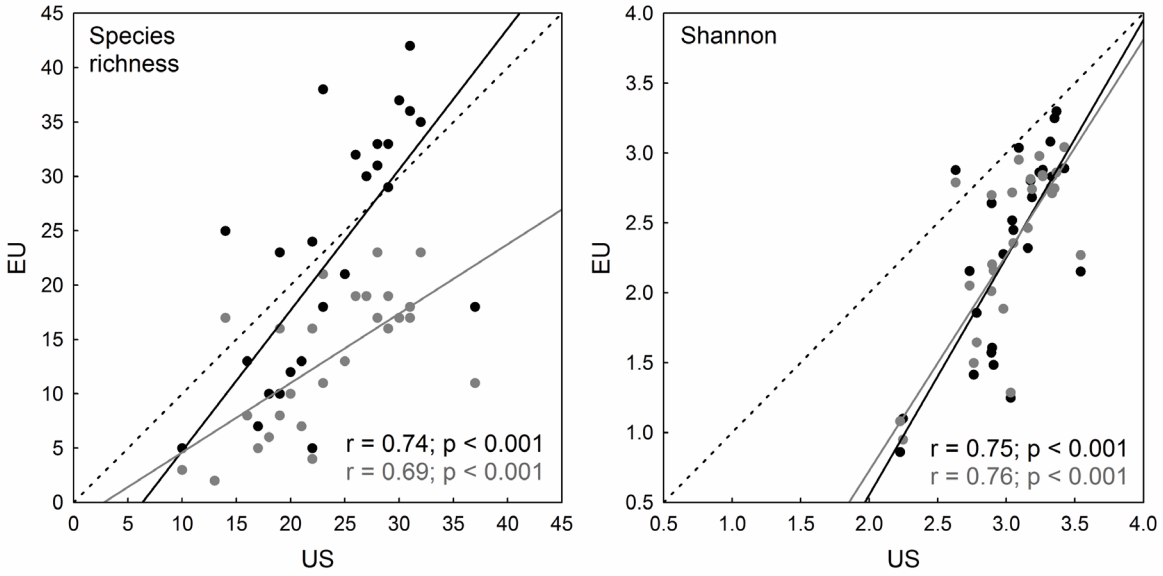

fig. 2. Taxonomic diversity indices computed using data collected with US and EU methods to assess epiphytic lichen diversity. Gray symbols represent EU macrolichens data and black symbols indicate EU data for all lichens. Numbers indicate Pearson correlation coefficients and significance. Solid lines are US vs. EU regression lines whereas dashed lines indicate 1:1 relationships were US and EU values are equivalent.

addition of a third axis yielded only a slight reduction in minimum stress. The final stabilities of the US, EU, and EUm ordinations were 7.04 %, 8.23 % and 7.86 %, respectively. Minimum stresses were lower than would be expected by chance for the three solutions ($p = 0.004$). Most of the variation in the datasets (US - 77.1 %, EU - 65.8 %, EUm - 61.4 %) was explained by axis one; axis two explained about 19% of the variation (18.7 %, 17.7 % and 20 %, respectively). Total variation explained was 95.8 %, 83.5 %, and 81.4 %, respectively.

Climatic and geographic groups detected by cluster analysis were also apparent in the NMS ordinations. US ordination was very similar to the EU (Mantel $r = 0.80$, $p < 0.001$) and to the EUm ordinations (Mantel $r = 0.81$, $p < 0.001$) ordinations, with information redundancies of 64 % and 65 %, respectively. US distance matrix was also highly associated with both EU matrices (both EU and EUm with Mantel $r = 0.81$, $p < 0.001$). EU ordinations were also very similar (Mantel $r = 0.98$, $p < 0.001$, redundancy of 96%), and their distance matrices were also highly correlated (Mantel $r = 0.97$, $p < 0.001$). Ordinations of the US, EU, and EUm lichen community composition extracted also the same two major macroclimatic gradients. The vector overlays in Figure 3 indicate correlating climate, pollution and functional group variables. The first axis represents a temperature and elevation gradient, evidenced by the high correlation coefficients of temperature and elevation with this axis (Table 2). The linear regressions between axis one scores and temperature almost completely overlap, emphasizing the similarity between methods. To a less

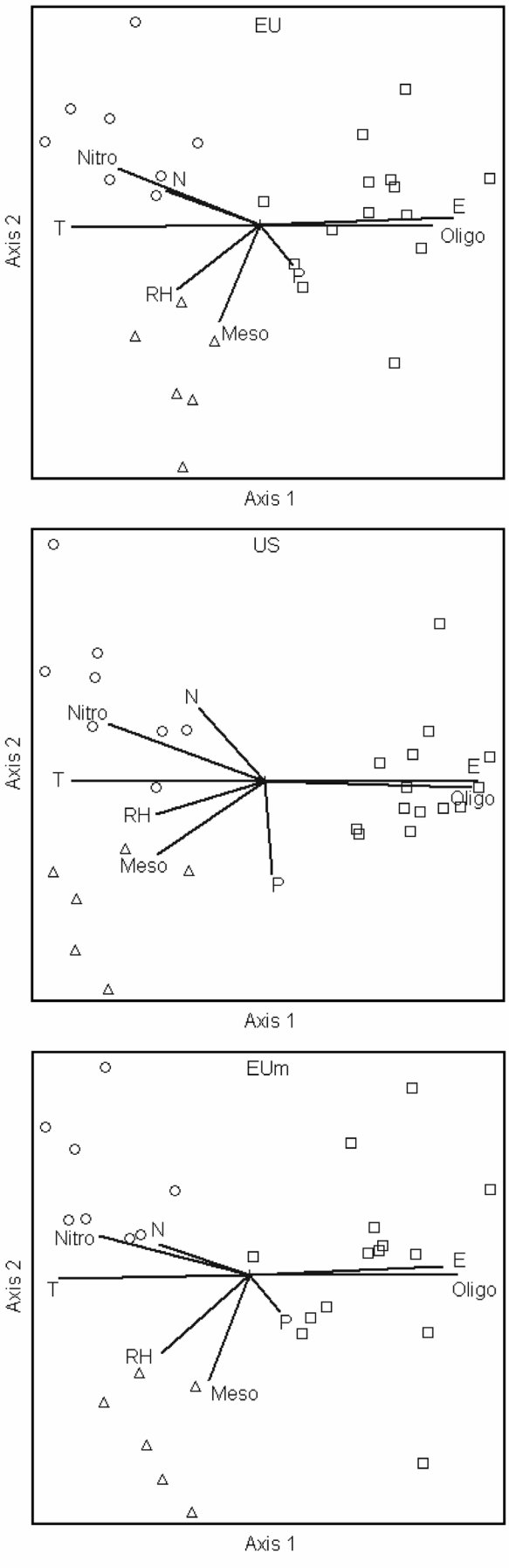

fig. 3. Non-metric multidimensional scaling (NMDS) ordinations of lichen community data from US and EU sampling methods. Vector overlays indicate the magnitude and direction of correlations between environmental variables and the ordination (see also Table 1). Climate variables: T - annual mean temperature, P - annual precipitation and E - elevation. Pollution variable: N - Dry weight N concentrations in lichens. Functional diversity variables: Nitro - CWM of nitrophylic species, Meso - CWM of mesophylic species and Oligo - CWM of oligophilic species. Symbols represent the model climatic and geographic areas defined by cluster analysis: circles - eastern Columbia River Gorge, triangles - western Columbia River Gorge and squares - high elevation Wilderness Areas.

table 2. Pearson correlations coefficients between ordination axes of lichen community data from US and EU sampling methods and climate, pollution and functional diversity variables. Significant correlations are marked: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

			US		EU		EU (macro)	
Type	Variable	Code	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Climate	Elevation	E	0.98***	0.01	0.93***	0.18	0.93***	0.20
	Annual mean temperature	T	-0.93***	0.01	-0.92***	-0.11	-0.93***	-0.12
	Annual precipitation	P	0.178	-0.64***	0.38*	-0.43*	0.37	-0.41*
	Relative humidity	RH	-0.70***	-0.39*	-0.61**	-0.54**	-0.63***	-0.59**
Pollution	Nitrogen deposition	N	-0.55**	0.58**	-0.65***	0.39*	-0.64***	0.34
Functional diversity	Nitrophilic	Nitro	-0.84***	0.51**	-0.80***	0.50**	-0.82***	0.42*
	Mesophilic	Meso	-0.69***	-0.58**	-0.42***	-0.66***	-0.43*	-0.69***
	Oligophilic	Oligo	0.96***	-0.17	0.88***	-0.06	0.97***	0.03

extent, this axis represents also a relative humidity gradient, slightly stronger on the US method ordination (Table 2). In all the ordinations, the second axis represents a moisture gradient of precipitation and relative humidity (Table 2). Nonetheless, with the US method, this gradient is more strongly related to precipitation, while with the EU method (both EU and EUm) relative humidity is slightly stronger. Similar pollution gradients were predicted by ordinations of all three datasets. Lichen nitrogen content was significantly correlated with both axes, except for axis 2 of EUm ordination (Table 2). Nonetheless, while for the US method this pollution gradient was almost equally distributed by both axes, in the EU method the gradient was more strongly reflected on the first axis of the ordination.

Lichen functional groups indicating tolerance to nitrogen pollution were highly correlated with the ordinations in both survey methodologies (Table 2). Individual correlations of CWM of lichen functional and climate and pollution variables show that US and EU methodologies give the same response trend, although with slight strength differences (Table 3). The sole exception was related with the relationship between CWM of nitrophilic species and relative humidity, which was only significant with the US method.

discussion

For the first time we show that results from lichen community data collected with the two most widely used survey methodologies in Europe and North America are highly comparable, using broadly accepted metrics of biodiversity to measure the effects of global change drivers. These findings are very promising, and ‘open the door’ to joint analysis of datasets from both continents to assess

table 3. Pearson correlations coefficients between community weighted mean (CWM) of lichen functional groups related to tolerance to nitrogen pollution obtained with US and EU sampling methods and climate, pollution variables. Nitro - nitrophilic, Meso - mesophilic and Oligo - oligophilic. Significant correlations are marked: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

		US			EU			EU (macro)		
Type	Variable	Nitro	Meso	Oligo	Nitro	Meso	Oligo	Nitro	Meso	Oligo
Climate	Elevation	-0.80***	-0.71***	0.95***	-0.69***	-0.58**	0.82***	-0.72***	-0.54**	0.95***
	Annual mean temperature	0.79***	0.62***	-0.90***	0.71***	0.51**	-0.82***	0.73***	0.47*	-0.93***
	Annual precipitation	-0.43*	0.15	0.25	-0.57**	0.28	0.30	-0.58**	0.30	0.32
	Relative air humidity	0.41*	0.79***	-0.64***	0.14	0.79***	-0.55**	0.20	0.76***	-0.61**
Pollution	Nitrogen deposition	0.74***	0.04	-0.62***	0.79***	-0.08	-0.56**	0.74***	-0.10	-0.60**

temporal and spatial global trends in lichen diversity.

COMPARABILITY OF METHODOLOGIES WITH DIFFERENT BIODIVERSITY METRICS

Regarding taxonomic diversity measures, species richness, Shannon-Wiener and Simpson's indices at individual survey sites values were highly correlated, depicting the same trends across the study area and allowing an inter-calibration. Further, the US dataset resulted in slightly higher values for both Shannon-Wiener and Simpson's indices, i.e. species abundances tended to be more similar across plots, and there was a higher likelihood of finding the same species across plots. Differences observed in the absolute values obtained with both methods would be expected from the differences in sampling methodologies resulting both from the detailed paid to inspect lichens in each method and to the different scales to rate their abundances. More importantly, despite these differences, our results show that both methods describe the same trends in response to climate and pollution gradients. Pielou's evenness index was the only index weakly related between US and EU datasets. This index measure can be problematic as it is based on the ratio of the sample-size independent Shannon-Wiener index and species richness, a more variable measure that is strongly dependent on sample size (Hurlbert 1971; McCune, Grace & Urban 2002). We do not recommend relying on Pielou's evenness measures for joint analyses of datasets derived from both protocols. However, we note that the Shannon-Wiener index is in part a measure of evenness and can be used to partly overcome this limitation (McCune *et al.* 2002).

The assessment of biodiversity shifts using community composition provided by both methods yielded even more striking similarities. The cluster analysis assigned the same sites

to the same three major biogeographical and climate/pollution groups independent of survey methodology. The community composition from both datasets described also the same trends along pollutions and climate gradients. Slight differences were observed in the strength of these gradients between both methods, nonetheless, they still classified sites almost identically, as shown by the almost overlapping scores. This striking similarity between methodologies is very important under a global change perspective. Shifts in species composition at local, regional and global scales in response to global change drivers have been observed (Dornelas *et al.* 2014; Savage & Vellend, 2014), and these results highlight the benefits that can be derived from collectively analysing data from both methodologies henceforth.

The community weighted mean of functional groups related to nitrogen deposition at each site was similar across datasets and trended similarly along the nitrogen gradient. These results are very important since these functional groups were already used separately in both continents to establish the lowest critical levels of atmospheric ammonia (Cape *et al.* 2009; Pinho *et al.* 2009) and total nitrogen deposition (Pardo *et al.* 2011; Root *et al.* 2015), a decisive contribution for environmental policies protective of ecosystem functions and services. Additionally, functional diversity has been a key concept in the assessment and interpretation of community response to environmental change (Lavorel *et al.* 2011), with regard to both air pollution and climate (Pinho *et al.* 2008, Matos *et al.* 2015). As it is not linked to species identity, functional diversity can be potentially more universal across broad geographical scales (Branquinho *et al.* 2015), illustrating at the same time the community structure. Our work reinforces this and shows that it can be a new way to explore and jointly analyse cross-continental data from distinct survey methods.

SPECIFICITIES AND CAVEATS OF EACH METHODOLOGY

The detail paid in inspecting the microlichen community appears to compensate for the lower number of trees measured using the EU method, thus resulting in higher species counts. Further, the different measures of species abundance (US - abundance ratings follow a log-like scale with only four choices; EU - allows a finer, more precise quantification of frequency but fewer trees sampled) account for the differences in Shannon and Simpson's indices. In general, macrolichens alone provided similar information about trends along climate and pollution gradients as macrolichens plus microlichens. This was previously shown in Europe for land use and climate gradients, where macrolichen diversity alone, when compared to total diversity assessed using

the EU method, was able to give the same response (Bergamini *et al.* 2005; Bergamini *et al.* 2007). Our results show that this is also true for climate and pollution. In this work, macrolichens accounted for around 50 % of the total number of species found using the EU method, or 31 % more species than the US macrolichens only method. Incorporating microlichens might be an advantage of the EU method in climates where macrolichens are less abundant than microlichens, e.g., closed tropical forests (Koch *et al.* 2013) or dry Mediterranean woodlands (Matos *et al.* 2015). These advantages can be weighted against extra costs incurred for field observer training and laboratory identification of the microlichens (Bergamini *et al.* 2007).

The EU survey protocol calls for a complete examination of a standard number of grid cells, using a limited number of phorophytes species (in this case a maximum of two per site), on a variable number of trees (usually between 4 and 10, determined by the environmental problem) and is not time constrained. The US method does not expect field observers to examine every tree, only to survey the range of substrates and microhabitats on the plot with a two-hour time constraint. Given the practical time restraint to keep up with the US field crews during this study, the average number of trees tallied by the EU method observer was six. Therefore, if anything, our work may underestimate the comparability of the data generated by the US and EU survey methods. Additionally, our study covered a limited environmental range relative to global change ranges. Future work should explore additional forest types and larger environmental gradients in terms of climate and pollution, to strengthen the robustness of the comparison and calibration between EU and US methodologies.

TOWARDS A UNIVERSAL FRAMEWORK

This works shows that, despite very different protocols, lichen survey data from EU and US methodologies can yield highly similar taxonomic and functional biodiversity metrics and community shifts along two major drivers of environmental change (nitrogen deposition and climate) and can be analysed jointly. We recommend that future cross-continental comparisons of EU and US datasets to track global change effects should be done using spatial and temporal trends of these three metrics, as suggested in our conceptual framework in Figure 5. A global biodiversity monitoring network is being developed with the purpose of assessing the impact of global change drivers on ecosystems (Pereira & Cooper 2006; Scholes *et al.* 2012). To enable retrospective and future analysis, the inclusion in this monitoring network demands that data collection is based on

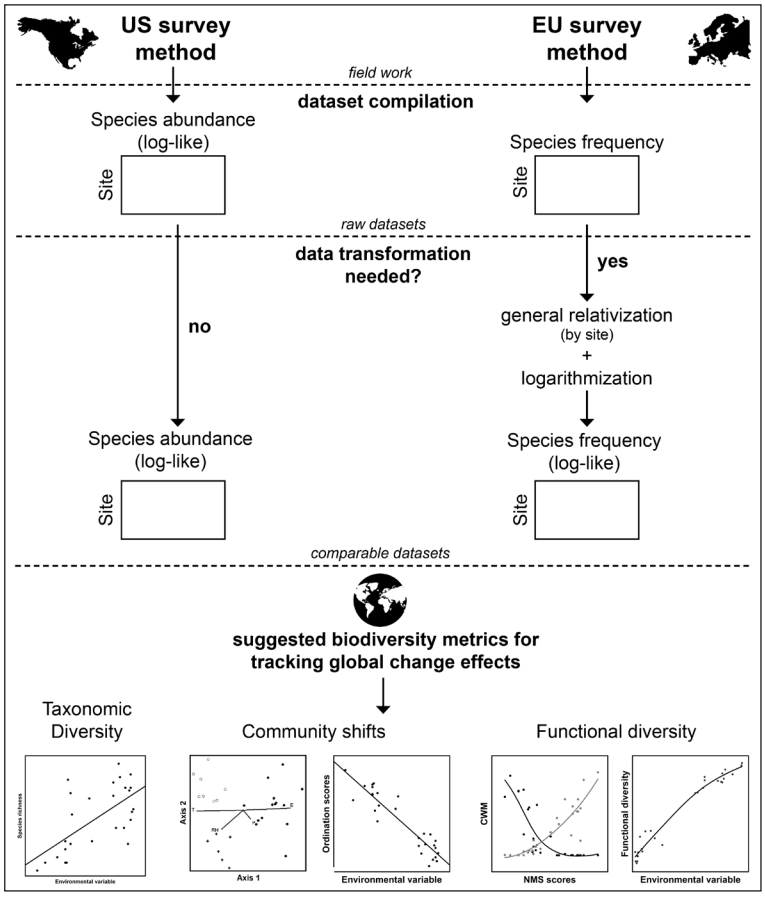

fig. 5. Conceptual framework for joint analysis of US and EU lichen diversity datasets for tracking global change.

compatible standardized methodologies performed at regional and global scales, and if responsive metrics are used to analyse the resulting data (Mace *et al.* 2005, MEA, 2005, Pereira & Cooper, 2006). However, this is only possible if standardized methodologies are applied at worldwide spatial and long-term temporal scales. The EU and US methods are used at a continental scale (North America and Europe) and could be used for global trend analysis at these continental scales. This framework confirms these methods compatibility and takes us one step closer towards lichens inclusion in this monitoring network. Furthermore, this framework integrates measures of taxonomic diversity, community structure and functional diversity and these are essential to more thoroughly comprehend ecosystems response to global change (Thuiller *et al.* 2006; McGill *et al.* 2015). Taken together, these results emphasize lichens potential to be used as large-scale integrated ecological indicators of global change.

EU lichen frequency values must be relativized prior to these comparisons, and its log transformation improves data comparability. Pielou's evenness index should be excluded from comparisons, as indicated by our results.

acknowledgments

We thank the US Forest Service International Volunteer Program for facilitating this collaboration, and Bruce McCune for advice on identification and analysis. This work was supported by the USFS Pacific Northwest Region Air Resource Management Program and FCT-MEC through: project PTDC/AAG-GLO/0045/2014; P.M. by contract SFRH/BD/51419/2011; P.P. by contract SFRH/BPD/75425/2010; A.N. by contract SFRH/BD/51407/2011; C.B. by contract Investigador FCT.

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chapter three

Lichen traits responding to aridity

publication

Matos P., Pinho P., Aragón G., Martínez I., Nunes A., Soares A.M.V.M., Branquinho C. (2015) Lichen traits responding to aridity. Journal of Ecology. 103(2), 451-458.

abstract

Climate change is expected to cause several impacts at the global scale, and drylands will be amongst the most affected areas. Thus, investigating how these changes will affect the composition, structure and functioning of dryland ecosystems has become a priority. From an ecological indicator point of view, several works have shown that functional diversity is better than species richness to understand ecosystem functioning or response to environmental factors. However, most of these works focus on plants, while those of other organisms remain largely unknown. Lichens are amongst the ecosystem components more sensitive to climatic changes due to several physiological and ecological characteristics. Their poikilohydric nature (therefore highly dependent on the atmosphere for water supply) and their ubiquity on terrestrial ecosystems underlie their potential as indicators of climate. Nonetheless, works specifically aiming to identify lichen functional traits that respond to aridity remain poorly explored, particularly in drylands.

We proposed to identify lichen functional traits and respective functional groups responding to aridity in a Mediterranean drylands ecosystem.

Lichen diversity was sampled in open holm oak woodlands along an aridity gradient in southwestern Europe (Iberian Peninsula). Lichen functional traits that could be easily identified and related to water uptake were selected to be tested: type of photobiont, growth form and reproduction strategy.

Lichen species composition was related to the aridity gradient. The three traits chosen were related with the community's response to aridity, but with contrasting responses in different functional groups. More specifically crustose and fruticose lichens, isidiate species and the ones with *Trentepohlia* as photobiont were related to the less arid part of the gradient. Foliose species and cyanolichens, on the contrary, were associated with the most arid areas.

We were able to identify lichen traits responding to aridity. Type of photobiont was particularly responsive, with *Trentepohlia* and cyanobacteria functional groups, responding clearly in contrasting ways to aridity in this drylands ecosystem. This work emphasizes functional diversity role on understanding and assessing the response to environmental factors, namely to climate. It also highlights the potential use of lichen functional groups as ecological indicators of climate change.

keywords

Determinants of plant community diversity and structure, drylands, functional response groups, growth form, photobiont type, reproduction type, semi-arid.

introduction

Drylands cover 41 % of terrestrial ecosystems (Reynolds *et al.* 2007) and concentrate around 38 % of world population (IPCC 2007). Within dryland ecosystems, semi-arid and dry–subhumid together share 29.7 % of world population representing 23.9 % of land surface (IPCC 2007), with dryland rangelands accounting for 50 % of the world's livestock (MEA 2005). These extremely important socio-ecological areas (Maestre, Salguero-Gomez & Quero 2012) are highly susceptible to climate change (IPCC 2007). For this reason, research on how global change will affect the composition, structure and functioning of dryland ecosystems and how in turn these changes will impair the wide range of services they provide (as the example given for livestock) and which support people livelihood and well-being has been considered a priority by scientific community (IPCC 2007).

From an ecological indicator perspective, several works have shown that functional diversity is better than species richness to understand ecosystem functioning or response to environmental factors, as it takes into account the redundancy of species in ecosystems and includes the information of species functional traits (Lavorel *et al.* 2011). Functional traits are characteristics of an organism considered to be relevant to its response to the environment and/or its effects on ecosystem functioning and its value and range in a given ecosystem are, by definition, a measure of its functional diversity (Díaz & Cabido 2001). Functional diversity is currently regarded as a crucial component within the ones affecting ecosystem services provision (Dıaz *et al.* 2006; de Bello *et al.* 2010). In fact, a framework linking response and effect traits – the response–effect framework – has been used to predict changes in ecosystem services (Lavorel & Garnier 2002). However, much of these promising results have been focusing on functional characteristics of plants, while those of other organisms remain largely unknown (Lavorel 2013).

Lichens are amongst the most sensitive organisms to environmental changes, signalling it before other less sensitive components of the ecosystems (Pinho *et al.* 2009, 2011, 2014). For that reason, they have long been used as ecological indicators. The use of lichen sensitivity to map air pollution (De Sloover & LeBlanc 1968), or to estimate SO_2 deposition (Hawksworth & Rose 1970) and the first signs of SO_2 decay (Rose & Hawksworth 1981), are just some of the striking examples of its historical use as ecological indicators of air pollution. Currently, works have also shown that they respond to global warming (Aptroot & Van Herk 2007; Ellis *et al.* 2007; Colesie *et al.* 2014). This sensitivity is related to their physiological characteristics. Lichens are a symbiotic association between a fungus and a photosynthetic partner and exist in a variety of land ecosystems, ranging from Antarctica to the deserts. These organisms are poikilohydric,

meaning that they cannot regulate their water content, which fluctuates to be in equilibrium with the surrounding environment (Green, Sancho & Pintado 2011). This external water dependence ultimately regulates their physiological activity, as wet environmental conditions hydrate and activate them, while under dry environmental conditions, they become inactive (Green, Sancho & Pintado 2011). Even though they are usually considered as slow-growing organisms, this dependence on the atmosphere enables them to respond to a climate shift on a time interval as short as 5 years placing them within the most sensitive responding to climate change (Aptroot & Van Herk 2007), which usually considers a time-scale of around 30 years. Nonetheless, although some works have been carried out in cold regions (Antarctica mainly) relating temperature gradients with species richness of lichens and other poikilohydric organisms (hepatics and bryophytes) (Green *et al.* 2011) or using lichenometry (Sancho, Green & Pintado 2007) under the current context of climate change, very few works have used them specifically as indicators of macro and microclimate (Giordani & Incerti 2008; Pinho, Maguas & Branquinho 2010; Marini, Nascimbene & Nimis 2011; Giordani *et al.* 2012, 2013; Colesie *et al.* 2014). However, works specifically aiming to identify lichen functional traits that respond to aridity remain poorly explored, particularly in drylands.

The objective of this work was to identify lichen key functional traits and respective functional groups that respond to aridity in a drylands ecosystem. We focused on how lichen composition varied along an aridity gradient, and how lichen functional diversity responded to this environmental driver, trying to identify the most relevant response traits to this response [a set of organisms sharing similar responses to the environment (Díaz & Cabido 2001)]. This was performed by sampling epiphytic lichens in south-west Mediterranean Europe along an aridity gradient within the semi-arid and on the border of its transition to the dry–subhumid.

materials & methods

STUDY AREA

The study was carried out in southwestern Europe (Iberian Peninsula), comprising 54 sampling sites distributed along Portugal and Spain. Lichen diversity was always sampled in open holm oak woodland, known as Montado in Portugal or Dehesa in Spain. Montado is a man-shaped savanna like ecosystem (agro–forestry–pastoral system) with a sparse tree cover (30–100 trees per ha) dominated by evergreen oaks (holm oak, *Quercus ilex* L., and/or cork oak, *Quercus suber* L.) (Pereira & Da Fonseca 2003; Bugalho *et al.* 2011). Grasslands, fallows and cereal crops compose its understory and are sometimes scattered with

mixed shrub formations (Pereira & Da Fonseca 2003; Bugalho *et al.* 2011). Sampling was performed along an aridity gradient across the semi-arid and up to the dry–subhumid climate (Fig. 1). We considered the aridity index (AI) of the United Nations (Atlas 1992), representing the ratio of mean annual precipitation to mean annual potential evapotranspiration. According to the UNEP classification, drylands are tropical and temperate areas with an AI < 0.65 and subdivided into four classes: hyper-arid (AI < 0.05), arid (0.05 < AI < 0.20), semi-arid (0.20 < AI < 0.50) and dry–subhumid (0.50 < AI <0.65). The gradient in our work was established within the semi-arid and ranged between 0.3 and 0.5.

SAMPLING

Lichen epiphyte communities were sampled on holm oak (*Q. ilex* L.) trees following a standard protocol (Asta *et al.* 2002). In each of the 54 sampling sites, a plot was established of c. 50 m radius and a minimum of one and a maximum of 10 trees were sampled (according to the number of suitable phorophytes found at each plot), with a total of 345 trees. A 10 cm x 50 cm grid divided in four 10 cm squares was placed on the four main aspects of the trunk (N, E, S, W), and all lichen species occurring in the quadrats were identified, and the number of quadrats where each species appeared was registered as its frequency. The uppermost part of the sampling grid was placed at 1.5 m from the ground following a standard protocol (Asta *et al.* 2002) and that height was adjusted to a maximum of two metres height when the trunk at the desired height was not suitable for sampling. When the identification was not possible in the field, samples were collected and taken to the laboratory for identification.

LICHEN DIVERSITY

A total of 161 species were identified and classified according to three traits (see Table S1 in this paper Supporting Information). In southern Europe, most works based their functional groups classification on the Italian data base (Nimis & Martellos 2008), as there is no international data base with lichen species traits as in the case of plants (Kattge *et al.* 2011). This data base classifies species into response traits (e.g. eutrophication tolerance or light preferences) according to expert knowledge based on ecological performance and has been shown to work well for responses to nitrogen (Pinho *et al.* 2011). However, as it is a national data base, not all species of Europe are included. For this reason, to make trait analysis independent of expert judgment assessment and geographical area, we selected photobiont type, growth form and main type of reproduction (Table 1). These are easily

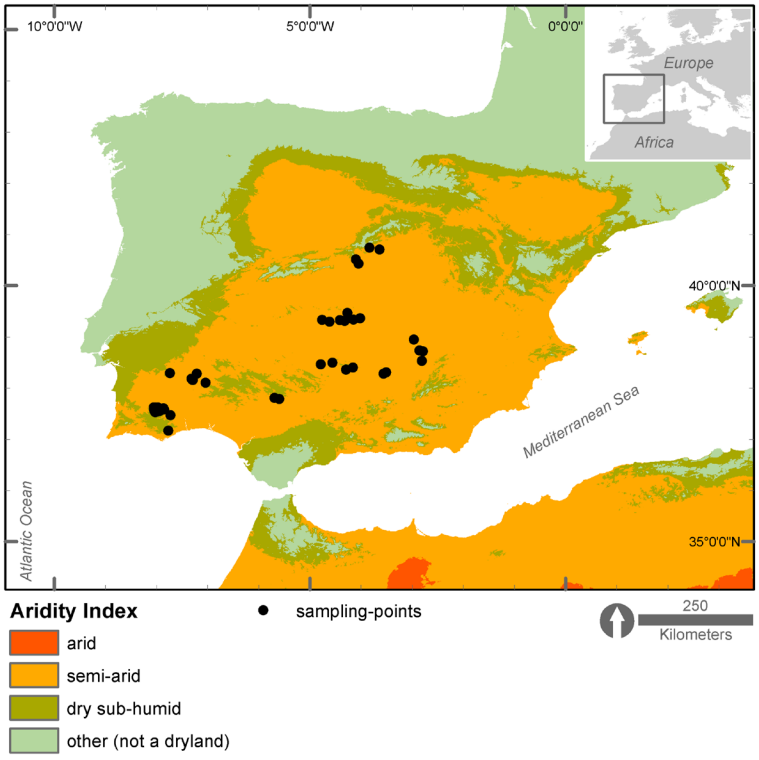

fig. 1. Map of the study area showing sampling sites and the aridity index (AI) gradient.

measurable lichen traits, that do not need identification to the species level, an important feature when there is a need for universal ecological indicators. Trait classification for each species was retrieved from the Italian lichen data base (Nimis & Martellos 2008). Information on species not present in this data base was taken from the Iberian Lichen Flora (Llop 2007; Giralt 2010; Carvalho 2012) and from The Lichens of Great Britain and Ireland (Smith *et al.* 2009).

Data were used to calculate the LDV index (Lichen Diversity Value) that accounts for species frequency (Asta *et al.* 2002), and it is presented as the mean value for each species of all the trees sampled per sampling site (sampling sites x species LDV). This species abundance measure was combined with species trait data to obtain the community level weighted mean (CWM) (Lavorel *et al.* 2008) (see Table 1 for traits and respective functional groups). This index represents the mean trait value (i.e. mean for each of its respective functional groups) in the community, weighted by the abundance of species having those values (Lavorel *et al.* 2008). It is actually the same as relative LDV calculated for groups of species sharing the same trait value. The 'dbFD' function implemented in the FD package (Laliberte & Legendre 2010) of CRAN software R (R Core Team 2013) was used to calculate the CWMs indexes.

table 1. Traits and related functional groups following (Nimis & Martellos 2008).

Trait	Functional group	Description	Symbol
Type of primary photobiont	Chloroccocoid	With Chloroccocoid (Green algae)	Ch
	Trentepohlia	With *Trentepohlia* (Green algae)	Tr
	Cyanolichens	With Cyanobacteria	Cy
Growth form	Crustose	Firmly and entirely attached to the substrate by the lower surface	Cr
	Crustose placodioid	Like crustose but with a plate-like form	Crp
	Leprose	Like crustose but surface thallus with a granular mass appearance and always decorticated	Lp
	Squamulose	Composed of small scales	Sq
	Foliose narrow-lobed	Partly attached to the substrate with a leaf-like form and narrow lobes	Fon
	Foliose broad-lobed	Same as foliose narrow-lobed but with broad lobes	Fob
	Fruticose	3D-like structure, attached by one point to the substrate with the rest of the thallus standing out from the surface of the substrate	Fr
	Fruticose filamentous	Same as fruticose but with filamentous form	Frf
Type of reproduction	Asexual sorediate	Mainly with soredia or soredia-like structures	As
	Asexual isidiate	Manly with isidia or isidia-like structures	Ai
	Sexual	Mainly sexual reproduction by spores	S

DATA ANALYSIS

Statistical analyses were performed using CRAN software R (R Core Team 2013) with VEGAN package (Oksanen *et al.* 2013) (functions 'metaMDS', 'envfit' and 'protest') and STATISTICA 11 (StatSoft, Tulsa, OK, USA). Non-metric multidimensional scaling (NMDS) ordination was performed on a matrix of sampling sites by species LDV to detect prominent gradients in species composition. When using data sets that include large gradients, species LDV values may vary greatly in absolute values due to local site characteristics unrelated to the environmental gradient of interest, biasing results and impairing comparisons. To solve this problem, species LDV values were relativized prior to the analysis and were used as % of total LDV of the sample, in a similar way as CWMs are calculated. Lichenicolous fungi and species identified only to genus level and without complete information on the three traits were excluded from the analysis. For the NMDS analysis, Bray–Curtis distance measure was used, as it has been shown to be one of the most effective measures of samples of species dissimilarities, and for this reason, the one recommended for community data (McCune, Grace & Urban 2002). Data underwent 500 iterations per run, and the best (lowest stress) solution from 500 runs with real data was chosen, each run beginning with a random configuration. The strength of the results was assessed comparing our resulting ordination with the ordination of 500 runs of randomized data (data randomized by column), using the 'protest' function that tests for the non-

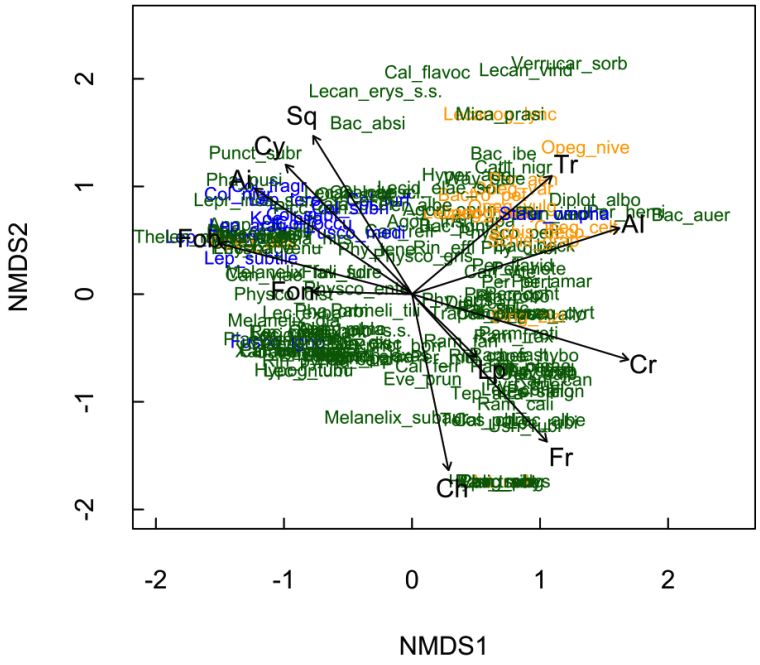

fig. 2. Non-metric multidimensional scaling (NMDS) analysis of species composition. Vectors represent significant correlations between community composition and environmental (AI) and functional variables (community level weighted mean). Only vectors with a significant correlation with the ordination ($P < 0.05$) and a significant Spearman correlation ($p < 0.05$) with individual axis 1 site scores (Table 2) are represented to prevent crowding: AI = Aridity index ($r^2 = 0.56$, $p = 0.001$); Tr = lichens with *Trentepohlia* ($r^2 = 0.45$, $p = 0.001$); Cy = cyanolichens ($r^2 = 0.45$, $p = 0.001$); Cr = crustose ($r^2 = 0.61$, $p = 0.001$); Fob = foliose broad-lobed ($r^2 = 0.47$, $p = 0.001$); Sq = squamulose ($r^2 = 0.52$, $p = 0.001$); Fr = fruticose ($r^2 = 0.56$, $p = 0.001$); Ai = Asexual isidiate ($r^2 = 0.47$, $p = 0.001$). Names indicate species' centroids (i.e. species scores along axes 1 and 2) and colours refer to the type of primary photobiont (Tr = orange, Gr = green, Cy = blue). (Final stress = 15.46, stress-based $R^2 = 0.98$, fit-based $R^2 = 0.89$.) First axis explains 42 % of the variability and the second 27 %.

randomness (significance) between two configurations. The coefficients of determination (r^2) between original plot distances and distances in the final ordination solution were calculated to assess how much variability in lichen community composition was represented by the NMDS axes (McCune, Grace & Urban 2002). AI and CWMs were overlaid in the NMDS ordination (McCune, Grace & Urban 2002), and significant correlations between community ordination and these variables were assessed using 1000 permutations (e.g. Jimenez *et al.* 2011). Correlation between individual NMDS site scores and AI and CWMs were also determined using Spearman correlations (ρ), to account for possible nonlinearity in the relationships (correlations were considered significant for $p < 0.05$).

results

The NMDS ordination joint plot shows species distribution in Fig. 2. This analysis suggested two axes (the addition of a third axis had only a slight reduction in minimum stress) with a final stability of 15.46. Minimum stress of the ordination was lower than would be expected by chance ($p = 0.33$). Most of the variability in lichen community structure was explained by axis 1 ($r^2 = 0.42$), whereas axis 2 explained less variability ($r^2 = 0.27$, Fig. 2). First axis site scores of the NMDS showed to be significantly correlated to the AI (Spearman $\rho = 0.67$, $p < 0.001$), which is assumed to be hereafter the main driver of species ordination. Axis 2 site scores were not correlated to the AI (Spearman $\rho = 0.14$) and because this was our environmental variable of interest, axis 2 was discarded from further analysis.

table 2. Summary of Spearman correlations (ρ) between non-metric multidimensional scaling ordination axis 1 and community-level weighted mean of trait values (functional groups). N = 54. Bold values represent significant correlations ($p < 0.05$).

Trait	Functional group	Symbol	ρ	*p*
Type of primary photobiont	Chloroccocoid	Gr	0.09	0.500
	Trentepohlia	Tr	**0.52**	**<0.001**
	Cyanolichens	Cy	**-0.42**	**0.001**
Growth form	Crustose	Cr	**0.81**	**<0.001**
	Crustose placodioid	Crp	0.22	0.120
	Leprose	Lp	0.18	0.202
	Squamulose	Sq	**-0.43**	**0.001**
	Foliose narrow-lobed	Fon	**-0.33**	**0.015**
	Foliose broad-lobed	Fob	**-0.70**	**<0.001**
	Fruticose	Fr	**0.48**	**<0.001**
	Fruticose filamentous	Frf	0.16	0.234
Type of reproduction	Asexual sorediate	As	0.14	0.327
	Asexual isidiate	Ai	**-0.57**	**<0.001**
	Sexual	S	0.24	0.080

Some functional group vectors (belonging to all the traits considered) were significantly correlated to the ordination solution and are shown in Fig. 2. Besides determining the correlation between functional groups and NMDS ordination, we also investigated the individual correlations between functional groups and NMDS axis 1 site scores, to establish the isolated effect of our main driver (Table 2). Concerning the type of photobiont, lichens with *Trentepohlia* and cyanolichens were associated to axis 1 of the ordination; the first ones associated to sites with lower aridity, while the opposite is observed for cyanolichens. Also the type of growth form responded to the gradient: crustose and fruticose species were associated to most humid area of the gradient, contrasting with foliose and squamulose species which were associated to the most arid areas. Regarding the type of reproduction, species with isidia showed to be related to the most arid part of the gradient.

discussion

Lichen species composition reflected the aridity gradient and allowed the identification of key traits and respective functional groups responding to this driver. Considering the photosynthetic strategy, different photobiont types showed different responses to aridity. Chloroccocoid algae lichens showed no correlation with species composition along the aridity gradient, probably due to the fact that 83 % (134 species) of the species found belong to this group and can be divided into the remaining traits and almost all of its respective functional groups, thus corresponding to a variety of functional traits combination that compromises its use as an indicator group for this range of aridity. On the other hand, lichens with *Trentepohlia* were associated with the less arid areas

of the gradient. These species have their optimum in shaded, warm–humid conditions, reason why they are mostly found in subtropical to tropical conditions (Nimis & Tretiach 1995). This combined sensitivity to relative air humidity and temperature may be the reason why they appeared in less arid sites. These sites are closer to the sea, under an Atlantic influence, thus characterized by higher air moisture contents, which favour *Trentepohlia* lichen species. In fact, the same pattern was found in a work that highlighted a photobiont-dependent response to climate in a large-scale pattern of epiphyte lichen species richness in Italy (Marini, Nascimbene & Nimis 2011). In their work, high species richness of *Trentepohlia* lichens was associated with warmer, wetter regions or under maritime influence, while continental areas sustained less species with this functional group, which almost disappeared in inner cold alpine regions (Marini, Nascimbene & Nimis 2011). Lichens having cyanobacteria as a photobiont were also correlated to the aridity gradient, but associated with the most arid areas. It is long known that cyanolichens need liquid water to activate photosynthesis (Lange *et al.* 1993; Green, Sancho & Pintado 2011) and also that they are regarded as highly sensitive to dry conditions (Pinho, Maguas & Branquinho 2010). In fact, it is not difficult to find references in literature strongly relating cyanolichens occurrence to sites with elevated air moisture contents (Jovan & McCune 2004). Still, most of these were carried out in temperate zones and with a large part of the species forming this functional group characterized as foliose broad-lobed (the emblematic macro cyanolichen species). Thus, one would not expect to find them associated to more arid conditions. However, several works have shown that not only they exist, but they are also common and ubiquitous in dry lands (Belnap, Budel & Lange 2001; Rogers 2006; Zedda *et al.* 2011; Giordani *et al.* 2013), and even increase with increasing aridity (Concostrina-Zubiri *et al.* 2014). The disparate results can be justified by the fact that cyanolichens associated to drier areas form a group of small-sized species with dark pigmentation (Zedda *et al.* 2011) that has been linked to semi-arid regions (Rogers 2006; Zedda *et al.* 2011; Giordani *et al.* 2013). In fact, a work three main hydration sources models for lichens (rain, dew and humid air) (Gauslaa 2014), showed forest cyanolichens responding to rain, as expected, and to a less extent to dew, this later ones corresponding spatially to arid regions. The extreme resistance of cyanobacteria to dry conditions has long been the subject of research, and even a book on the ecology Nostoc species, the one present in these dark coloured cyanolichens, is long known (Dodds, Gudder & Mollenhauer 1995; Seckbach 2007; Sand-Jensen & Jespersen 2012). Yet, besides the fact that the dark pigmentation can act as a protection mechanism (Gauslaa & Solhaug 2001; McEvoy, Gauslaa & Solhaug 2007)

and that their lower temperature limit is around 0 °C (Green *et al.* 2011), not much is known about the reasons underlying cyanolichens capacity to withstand higher temperatures, intensive solar radiations and lower atmospheric moisture contents. Further work is needed to understand why these dark pigmented cyanolichens respond in such a different way from the non-dark pigmented ones and what traits could be involved in this different behaviour. More importantly, this work highlights the importance of considering these two functional groups of cyanolichens separately in works dealing with the influence of climate.

Regarding growth form trait, crustose and foliose functional groups were associated to the aridity gradient, the crustose preferring lower aridity and the foliose (mainly the broad lobed but less pronouncedly the narrow-lobed ones) associated to higher aridity sites. The same pattern was found in Italy in response to rainfall gradients, where crustose species were found to be associated with plots with higher precipitation (Giordani *et al.* 2012). Also in Scotland, foliose species showed to become less frequent in stands from wetter localities, reflecting a climatic gradient across Scotland, from the wet and oceanic Atlantic seaboard to the drier and more continental north-east (Ellis & Coppins 2006). Fruticose species were associated to sites with lower aridity. This functional group is usually regarded as indicator of light-related factors caused by forest structure, like canopy openness, as light is usually their most limiting factor (Giordani *et al.* 2012; Li, Liu & Li 2013) and is commonly known to be bound to more humid conditions (Belnap, Budel & Lange 2001; Giordani *et al.* 2013). In our sampling sites, forest structure and thus canopy openness and light conditions were more or less even (all sites in Montado with similar structure). Thus, air moisture content in sites with higher aridity values (with an Atlantic influence) may be the determinant factor linking fruticose species as indicators of this part of the gradient.

Regarding the reproduction trait, only mainly asexual species with isidia showed to be associated with the aridity gradient. Isidia are corticated diaspores, variable in size and shape, whose dispersal is favoured by water run-off, as its heavy structure probably makes them less efficient for dispersal than soredia or spores (Giordani *et al.* 2013). Accordingly, most of recent genetic and population studies have focused on these species dispersal ability, establishment and survival (Scheidegger, Frey & Zoller 1995; Zoller, Frey & Scheidegger 2000). Although, to our knowledge, its relation with climate has not been directly addressed, a work with *Lobaria pulmonaria* (L.) (Martınez *et al.* 2012) showed a higher frequency and abundance of isidia upon tree trunks closer to the canopy, where microclimatic conditions are harsher (higher radiation and temperature, lower humidity).

Some authors have pointed out that important changes may occur in species with a dense cover of isidia (Jahns 1984). More isidia cover could modify thallus water absorption (Rikkinen 1997) and water-holding capacity (Tretiach *et al.* 2005) in a way similar to trichomas in plants. This may be a possible explanation for why these species appear associated with the most arid areas in our gradient, as this characteristic may represent an improvement of water absorption and water-holding capacity.

In conclusion, the three traits chosen showed to be relevant to the response to aridity. Type of photobiont appears particularly promising, namely *Trentepohlia*, as seen in previous works (Aptroot & Van Herk 2007), and cyanobacteria functional groups, responding clearly in contrasting ways to aridity. This work emphasizes once again the role of functional diversity on understanding and assessing the response to environmental factors, as previous works did (Lavorel *et al.* 2011) and brings a fresh new insight to this area of research from a less known group of organisms, a gap of knowledge that had been pointed out before (Lavorel 2013). This first community level approach based on traits that require minimum expert knowledge worked well, showing its potential to be further developed and applied in the future. Understanding key functional traits associated to aridity is a fundamental step to build up a model of ecological indicators of climate change for drylands (Maestre, Salguero-Gomez & Quero 2012). These results highlight lichen functional diversity potential for areas where shifts towards drier climatic conditions are expected to occur, opening new doors for future research in this area. Nonetheless, future work should focus on the search for other functional groups that may also be potential candidates of ecological indicators of climate change in drylands.

This work was limited to a single environmental variable, the AI, which has some limitations to its use as it does not account for other forms of precipitation such as mist, clouds, dew or fog, which could be more important in ecological terms for this group. These sources of hydration are all known to be important for lichens and connected to the patterns of functional variation in growth form and type of photobiont, as a recent work highlighted (Gauslaa 2014). Large-scale ecological studies have to rely on the available data at a large scale, and it is widely known and accepted that the existent precipitation models are limited in terms of spatial resolution, relative air humidity models are rarely available and models for dew, mist or fog are inexistent at a global scale. This work emphasizes that even using a variable with such limitations, it is possible to explain a significant part of the functional diversity pattern observed, revealing its potential for a wide-scale use. Nonetheless, future research on other functional traits and groups responding to aridity gradients should

also contemplate including, when available, other environmental variables known to be important in lichen ecology. Additionally, in this context of global change, an important challenge to future research will be to disentangle the effects of climate drivers from other major drivers of global change in drylands, such as nitrogen deposition, to access its independent effects and its interactions.

data accessibility

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.vn2sh (Matos *et al.* 2015).

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chapter four

Mediterranean lichen traits are primarily filtered by seasonal temperatures: a promising tool to track climate change

publication

Matos P., Giordani P., Pinho P., Aragón G., Martínez I., Nunes A., Soares A.M.V.M., Branquinho C. In preparation for submission to Global Ecology and Biogeography

abstract

Functional diversity is necessary to improve biodiversity predictions and to quantify ecosystem functionality in response to global change drivers. Although we already know which lichen traits respond to aridity in drylands, the specific climate filters influencing traits' responses remain unclear.

We evaluated which climate variables are driving lichen traits and functional groups looking at the role of temperature, precipitation and relative humidity. For that, lichen diversity was sampled in southern Europe following an aridity gradient.

Temperature related variables were the key predictors of lichen traits response. Interestingly, lichens response was not related to annual averages, but rather to temperature range and seasonal variance. The residual importance of precipitation on lichen trait filtering was also related with its seasonal variation, rather than its total amount. Lichen functional groups belonging to the same trait responded differently to different climatic variables.

The differential sensitivity of lichen functional diversity to a broad array of climatic variables, especially those forecasted to change, can be used to develop trait-based ecological indicators for better predictions in a context of climate change. Lichens have demonstrated responses to recent climate change, and our study shows that they have the potential to signal fine scale changes of seasonal climatic variations.

keywords

Climate seasonal and range variance, global warming, growth form, lichen response traits, Mediterranean, photobiont type, size

introduction

Drylands occupy 41 % of all terrestrial ecosystems, housing around one third of the world's population (MEA 2005). Unprecedented climate warming and changes in precipitation patterns over the last decades have already impacted natural and human systems in drylands, and these changes, which have a worldwide scale, are predicted to worsen in the near future (IPCC, 2013). In southern and central Europe, summer and daytime extremes are expected to raise faster than mean temperature, something that could be related to the forecasted increase in diurnal temperature range (Kirtman *et al.* 2013). On the other hand, summer and winter trends from 1950 to 2010 highlight differential seasonal patterns of change, and this trend may continue in the near future (Kirtman *et al.* 2013). Considering the high socio-ecological value of drylands, it is easy to understand why research on climate change and other global change effects on these ecosystems has been prioritized (Maestre, Salguero-Gomez & Quero 2012).

Several works documented phenological, distributional,

community composition or abundance changes attributable to climate change on a variety of organisms ranging from animals to plants, across marine and terrestrial ecosystems from all over the globe (some impressive meta-analysis: Parmesan & Yohe 2003; Parmesan 2006; Dornelas *et al.* 2014). This conspicuous change in biodiversity at the global scale has escalated to the point that by itself is already considered as a major global change driver (Hooper *et al.* 2012), resulting in profound ecological and societal consequences (Chapin III *et al.* 2000; MEA 2005; Cardinale *et al.* 2012). Facing these problems requires understanding and quantifying how ecosystems' functioning is responding to global change, and biodiversity metrics and other indicators are some of the fundamental tools to do it (Pereira *et al.* 2013).

During many years, species richness was the metrics of choice to quantify ecosystems change in response to environmental drivers. However, species-related indices perform poorly when measuring global change drivers (Dornelas *et al.* 2014). This is likely because despite the expansion in the number of threatened species (Butchart *et al.* 2010), globally it does not trigger a downward trend in species richness and related indices, but rather a shift in species composition over time (Dornelas *et al.* 2014). If only species-related metrics are used, species redundancy in the ecosystems will not be accounted for when compositional shifts occur and comparison at larger geographical scales may be impaired due to species limited distributions. Functional diversity is a better indicator to quantify ecosystem functionality in response to global change drivers (Díaz & Cabido 1997; Díaz *et al.* 2007; Suding *et al.* 2008; Lavorel *et al.* 2011). This functional trait approach is able to quantify compositional shifts accounting for species redundancy and has the potential to be both universal and applicable at broad spatial scales, because it is not linked to species per se. A functional trait is a characteristic of an organism that is relevant to its response to the environment and/or its effects on ecosystem functioning (Díaz & Cabido 2001). Its value and range in a given ecosystem are, by definition, a measure of functional diversity (Díaz & Cabido 2001).

Lichens are amongst the most sensitive organisms to environmental change, signalling it before other less sensitive components of the ecosystems (Pinho *et al.* 2009; Pinho *et al.* 2011; Pinho *et al.* 2014). This sensitivity to atmospheric change is the reason why they have been used for more than a century to track the major drivers of global change related to atmospheric pollution. Lichens respond also to climate change (Aptroot & van Herk 2007), and compositional shifts were observed in response to global warming in time intervals as short as 5 years (van Herk, Aptroot & van Dobben 2002). Lichen response traits

have successfully been used to track micro (Pinho, Máguas & Branquinho 2010; Giordani *et al.* 2012; Giordani *et al.* 2014) and macroclimate conditions (Giordani & Incerti 2008; Marini, Nascimbene & Nimis 2011; Colesie *et al.* 2014; Concostrina-Zubiri *et al.* 2014; Matos *et al.* 2015; Nascimbene & Marini 2015).

Functional traits enlighten us on how environmental factors shape biodiversity (de Bello *et al.* 2013), and are also thought to improve biodiversity predictions under global change (Suding *et al.* 2008). Nonetheless, before we can incorporate trait-based ecological indicators for better predictions in a context of climate change, we need first to clarify two basic steps. First, it is necessary to identify key functional traits associated to climate change. Second, we need to know which specific climate filters are influencing trait's response to better use them as ecological surrogates. This second step is fundamental to understand which climate variables drive lichen biodiversity patterns and those that should be kept under close attention in the future. Thinking about the sustained observations of ongoing and future climate change, a set of essential climate variables was defined in an effort to guide the implementation of the Global Climate Observing System by the Parties of the United Nations Framework Convention on Climate Change (UNFCCC). Yet, from the available set of essential climate and bioclimatic variables (WorldClim), we are still far from knowing exactly which are the essential ones driving changes in biodiversity patterns, and at which should temporal sustained observations be focused on. Recent advances have taken us one step closer to this, and we have now a set of lichen response traits with recognizable responses to aridity in Mediterranean drylands (Matos *et al.* 2015). However, though essential, this first approach was based on a climate variable that integrates several others, and we still need detailed information on the specific basic climate filters acting on these traits and their respective functional groups. This may be particularly relevant in the highly seasonal Mediterranean region, where annual averages of temperature and precipitation do not reflect the dramatic seasonal changes that occur year round.

The objective of this work was to understand which specific climate filters act on epiphytic lichen response traits and respective functional groups in Mediterranean drylands ecosystems. We focused on the most important available climate variables that could be acting on lichen functional diversity variation along an aridity gradient, based on lichen traits identified as responsive in this region (Matos *et al.* 2015). The Mediterranean region has a markedly seasonal climate, in general characterized by low and highly variable precipitations patterns, usually concentrated in the cold season, and dry hot summers. We aim to understand

not only which climate variables are primarily driving lichen traits and functional groups (precipitation, temperature, relative humidity or a composed index like aridity), but also to tackle the role of temperature and precipitation variance along the year (e.g. seasonal and daily or annual range). This work will help us understand which climate filters shape lichen trait composition, simultaneously updating us about those that should be tracked under a context of climate change when using lichen functional diversity as an ecological indicator. Furthermore this work will help us comprehend if lichens can in fact be used as ecological indicators to track climate change. For that lichen epiphyte diversity was sampled in southern Europe along an aridity gradient ranging from the semi-arid to the hyper-humid.

materials & methods

STUDY AREA

The study area was located in Mediterranean SW Europe distributed across Portugal (n = 33), Spain (n = 25) and Italy (n = 15) (Fig. 1), totalling 73 sites in holm oak (*Quercus ilex* L.) woodlands. Sampling was done following an aridity gradient ranging from the semi-arid to the dry sub-humid and going over temperate regions, based on the aridity index (AI) of the United Nations (Middleton & Thomas 1992). This index represents the ratio of mean annual precipitation to mean annual evapotranspiration. According to the UNEP classification, drylands are tropical and temperate areas with an AI < 0.65 and sub-divided into: hyper-arid (AI < 0.05), arid (0.05 < AI < 0.20), semi-arid (0.20 < AI < 0.50) and dry–subhumid (0.50 < AI < 0.65). Places with an aridity index between 0.65 and 0.75 are considered humid, while those with an aridity index higher than 0.75 are classified as hyper-humid regions. Our gradient ranged from Mediterranean semi-arid drylands (AI = 0.30) with average minimum and maximum annual temperatures of 2.8ºC and 34ºC, respectively, and 360 mm annual precipitation, to a hyper-humid region (AI = 1.6) with average minimum and maximum annual temperatures of -3ºC and 20ºC, respectively, and around 993 mm annual precipitation.

LICHEN SAMPLING

Epiphytic lichen communities were sampled at these 73 sampling sites on holm-oak (*Quercus ilex*) trees following a standard protocol (Asta *et al.* 2002). In each sampling site a minimum of 1 and a maximum of 10 trees were sampled (depending on the number of suitable phorophytes found at each plot, total trees N = 403). A 10 cm × 50 cm grid divided in four 10 cm squares was placed on the four cardinal aspects of the trunk. The uppermost part of the sampling grid was placed at 1.5 m from the ground as indicated in the standard protocol (Asta *et al.* 2002). That

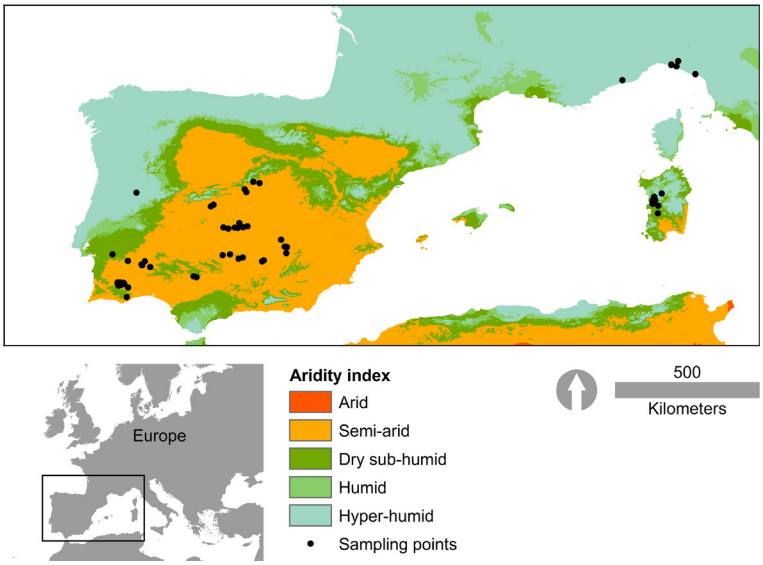

fig. 1. Map of the study area showing sampling sites and the aridity index (AI) gradient.

height was adjusted to a maximum of 2 m whenever the trunk at the desired height was not suitable for sampling. All lichen species occurring in the quadrats were identified and the number of quadrats where each species appeared was registered as its frequency. A total of 216 species were identified (supplementary table 1). When impossible to identify in the field, lichen samples were collected and taken to laboratory for identification.

CLIMATIC DATA

A collection of 21 climate variables were selected for the analysis and extracted for the 73 sampling sites. Nineteen bioclimatic variables were taken from WorldClim representing the mean, range and seasonal variation of temperature and precipitation (table 1). These climate data correspond to average monthly climate data from 1960 to 1990 from weather stations, interpolated on a 30 arc seconds resolution grid (see www.worldclim.org for more details on variables with codes Bio 1 to Bio 19). As this work was done following an aridity gradient, the global aridity index was also extracted and included in the set (http://www.cgiar-csi.org/data/climate/item/51-global-aridity-and-pet-database). This data is available at the same spatial resolution as precipitation and temperature variables, and was modelled using the available data from WorldClim. Finally, as lichens are known to respond to other sources of hydration such as relative air humidity (Gauslaa 2014), this variable was also included in the set (extracted from the Biosphere Atlas and modelled with original data from Climate Research Unit also at a resolution of 30 arc seconds (New *et al.* 2002). To facilitate the interpretation of results, bioclimatic variables related to temperature and precipitation were grouped into annual mean, range (daily, annual and isothermality), and

table 1. Climate variables used in the study.

Main variable	Type		WorldClim code	Variable
Temperature (ºC)	Annual		Bio 1	Annual Mean Temperature (AMT)
	Range		Bio 2	Mean Diurnal Range (MDR)
			Bio 7	Temperature Annual Range (TAR)
			Bio 3	Isothermality (I)
	Seasonal	Seasonality	Bio 4	Temperature Seasonality (TS)
		Summer	Bio 9	Mean Temperature of Driest Quarter (MTDQ)
			Bio 10	Mean Temperature of Warmest Quarter (MTWMQ)
			Bio 5	Maximum Temperature of Warmest Month (MAXT)
		Winter	Bio 8	Mean Temperature of Wettest Quarter (MTWTQ)
			Bio 11	Mean Temperature of Coldest Quarter (MTCQ)
			Bio 6	Minimum Temperature of Coldest Month (MINT)
Precipitation (P)	Annual		Bio 12	Annual Precipitation (AP)
	Seasonal	Seasonality	Bio 15	Precipitation Seasonality (PS)
		Summer	Bio 17	Precipitation of Driest Quarter (PDQ)
			Bio 18	Precipitation of Warmest Quarter (PWMQ)
			Bio 14	Precipitation of Driest Month (PDM)
		Winter	Bio 16	Precipitation of Wettest Quarter (PWTQ)
			Bio 19	Precipitation of Coldest Quarter (PCQ)
			Bio 13	Precipitation of Wettest Month (PWT)
Global aridity index				Annual Mean (AI)
Relative humidity				Annual mean (RH)

seasonal variation (seasonality, summer and winter; Table 1).

LICHEN FUNCTIONAL DIVERSITY

In this work we focused on three response traits and their respective functional groups. Trait classification was retrieved from the Italian database (Nimis & Martellos 2008), or, if absent from that database, from lichen floras (Llop 2007; Smith *et al.* 2009; Giralt 2010; Carvalho 2012). Photobiont type and growth form (see Table 2 for respective functional groups) were chosen due to their known responsiveness to aridity and potential to be used as climate change indicators (Matos *et al.* 2015). Additionally, a third trait was added based on a simple lichen size classification mainly driven by growth form. Lichens were classified as microlichens when crustose or leprose, and as macrolichens if otherwise.

Epiphytic lichen data was used to calculate species frequencies

table 2. Traits and related functional groups (Nimis & Martellos, 2008; Smith *et al.* 2009).

Trait	Functional group	Description	Symbol
Type of primary photobiont	Chlorococcoid	With Chlorococcoid (Green algae)	Ch
	Trentepohlioid	With *Trentepohlia* (Green algae)	Tr
	Cyanolichens	With Cyanobacteria	Cy
	Resistant cyanolichens	A specific set of lichens with cyanobacteria and jelly structure (homoiomerous), that are mostly found in dry climate.	Cyj
Growth form	Crustose	Firmly and entirely attached to the substrate by the lower surface	Cr
	Leprose	Like crustose but surface thallus with a granular mass appearance and always decorticated	Lp
	Squamulose	Composed of small scales	Sq
	Foliose narrow-lobed	Partly attached to the substrate with a leaf-like form and narrow lobes	Fon
	Foliose broad-lobed	Same as foliose narrow-lobed but with broad lobes	Fob
	Fruticose	3D-like structure, attached by one point to the substrate with the rest of the thallus standing out from the surface of the substrate	Fr
Size	Microlichens	With crustose or leprose growth form.	Mi
	Macrolichens	With growth form other then crustose and leprose	Ma

(Asta *et al.* 2002), presented here as the mean value for each species of all the trees sampled per sampling site (sampling sites × species frequency). Community level weighted mean (CWM) was calculated combining species abundance and trait data (Lavorel *et al.* 2008) using 'dbFD' function implemented in the FD package (Laliberté & Legendre 2010) of CRAN software R (R Core Team 2013). Because we only have categorical traits, the CMW represents the mean proportion of each functional group (FG) in the community, weighted by the abundance of species belonging to those functional groups (Lavorel *et al.* 2008). This resulted in a matrix with the CWM of all functional groups of these three traits for each sampling site (CWM of each FG × site).

DATA ANALYSIS

Spearman correlation coefficients were calculated between climate variables and lichen functional groups CWM to account for possible non-linearity (correlations were considered significant for $p < 0.05$). For functional groups having significant correlations and exhibiting a linear pattern, we calculated general linear models (GLM) including all the significant variables, ensuring that if variables were collinear only one was chosen at a time to be included in the model. GLM analyses were done with Statistica software version 12. From all the models returned by the software, a rule of maximum R^2 with the smallest number of predictors was followed to select the best ones. Models with more than one predictor were kept if its R^2 increased by more than 5% relatively to the simplest one. Additionally, when competing models (same number of predictors) with different combinations

of predictors showed similar R^2 values, the first three models within 5% R^2 values from the highest one were considered.

For functional groups showing non-linear responses to climate, we ran a non-parametric multiplicative regression (NPMR, McCune 2006) in Hyperniche version 2.28 (MjM Software Design, Gleneden Beach, OR, US). This method accounts both with complex linear and non-linear relationships between functional diversity and climatic variables and with multi-factor responses of lichen functional groups due to interaction between predictive variables. A local mean estimator was used with a Gaussian weighting function in a forward stepwise regression, simultaneously optimizing smoothing parameters (tolerance). Minimum average neighbour size was set to three. Leave-one-out-cross-validation was used for over-fitting control (medium over-fitting was selected) and a cross-validated R^2 (xR^2) was calculated as a measure of fit. The relative importance of individual explanatory variables within the selected models was evaluated by a sensitivity analysis (McCune 2006). A sensitivity value of zero indicates that the predictor has no detectable effect on the response, while a sensitivity value of one means that on average, nudging that predictor results in a response change of equal magnitude. NPMR models were developed individually for each functional group and climate variable, and for all climate variables combinations. From all the models returned by the software, again a rule of maximum xR^2 with the smallest number of predictors was followed to select the best ones. Models with two predictors were only kept if its xR^2 increased more than 5% over the simplest one. A randomization test was used to assess model significance, comparing the estimation of selected models with an average estimation calculated by 1000 random permutations among the dataset. In both types of model, GLM and NMPR, when competing models (same number of predictors) with different combinations of predictors showed similar xR^2 values, the first three models within 5% xR^2 or R^2 of the highest one were considered. To facilitate comparison of sensitivity between GLM and NPMR models, sensitivity of predictors in NMPR models was relativized by sum of all predictor's sensitivity in the model. Variables included in one-dimensional models, NPMR or GLM, were considered therefore to have a sensitivity of 1.

results

Five functional groups related to growth form and size showed strong linear correlations with the environmental variables and better than the non-linear ones. For these five functional groups all models were one-dimensional (Table 3). For the functional groups related with photobiont type and for the other growth forms, the best models where those with non-linear regressions. Models descriptions with each functional group best predictors

table 3. Best climate predictor models for lichen functional groups response. See table 1 for climate variables names and symbols, and table 2 for functional group names.

					NPMR							GLM	
Trait	Func. group	Var. 1	Var. 2	No. pred.	xR^2	Ave. size	Tol. 1	Tol. 2	Monte Carlo	Sens. 1	Sens. 2	R^2	p
Photo-biont	Ch	TS	MTCQ	2	0.42	8.3	148.92	3.44	< 0.001	0.53	1.81		
		TS	MINT	2	0.39	8.4	148.92	4.08	< 0.001	0.53	1.20		
	Cy	I	PS	2	0.80	8.9	0.34	3.52	< 0.001	2.64	0.40		
	Cyj	MTWMQ	PWTQ	2	0.76	8.9	3.19	30.20	< 0.001	1.31	0.26		
		MDR	MTWMQ	2	0.74	8.5	3.25	4.26	< 0.001	0.56	0.84		
		I	MTWMQ	2	0.72	8.3	1.01	3.19	< 0.001	0.45	1.36		
	Tr	TS	PCQ	2	0.37	7.2	49.64	42.31	< 0.001	0.68	0.09		
		I	TS	2	0.35	7.5	1.86	49.64	0.004	0.10	0.95		
		MDR	TS	2	0.34	7.4	7.13	49.64	0.002	0.11	1.13		
Growth form	Cr	I	MTDQ	2	0.58	9.6	0.34	9.78	< 0.001	3.24	0.32		
	Lp	MDR		1	0.55	10.3	1.30		< 0.001	1.99			
	Sq	AMT		1	0.19	20.7	4.92		0.002	0.55			
		PDQ		1	0.18	21.3	5.64		0.001	0.95			
		I		1	0.18	13.5	0.68		< 0.001	0.62			
	Fon	RH		1								0.56	< 0.001
	Fr	RH		1								0.17	< 0.001
	Fob	MTWTQ		1								0.20	< 0.001
Size	Mi	TS		1								0.54	< 0.001
		TAR		1								0.52	< 0.001
	Ma	TS		1									< 0.001
		TAR		1								0.52	< 0.001

are shown in Table 3 and Fig. 2. The response of functional groups to their best climate predictors is shown in Fig. 3. We did not find a common response of functional groups belonging to the same trait. Some functional groups showed stronger responses than others, but all functional groups were significantly predicted by some climatic variable.

Of the twelve functional groups, only two did not include temperature related variables. Overall, temperature related variables were the main predictors of lichen functional groups response in terms of number of models represented, and in terms of sum of sensitivities in the models. Annual mean temperature was only important for squamulose species and in a model with a fit lower than 0.20. On the other hand, temperature range variables were the overall most important ones in terms of number of models represented, being absent only from two growth form functional groups models, fruticose and foliose narrow lobed. Temperature range were the only variables explaining lichen size

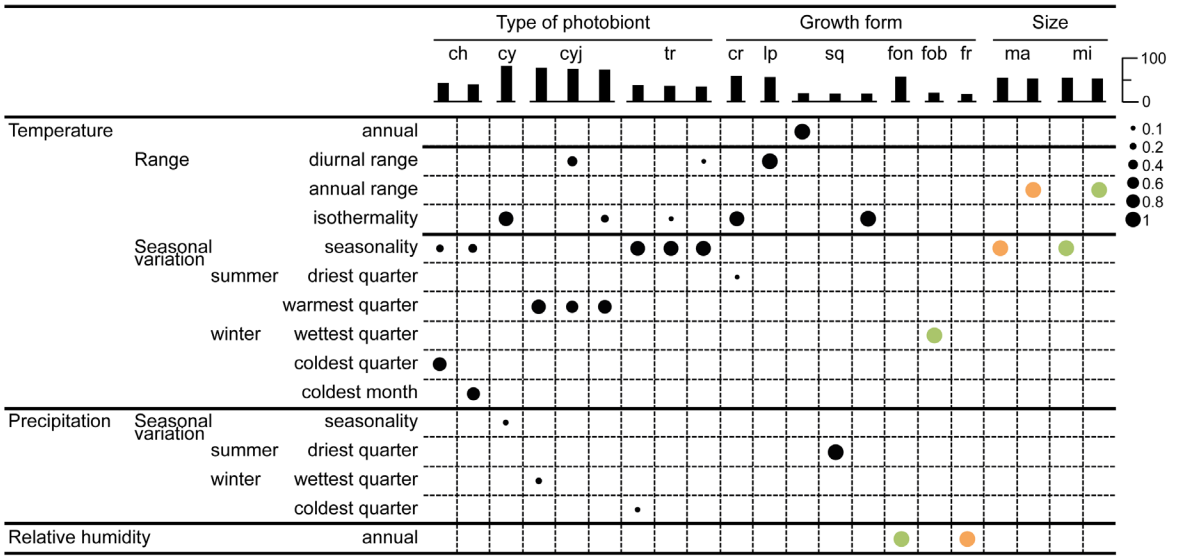

fig. 2. Climate variables selected as best predictors in each functional group model. Models depicted by black circles represent non-linear regressions (NPMR) and coloured circles represent linear regression (GLM; green circles stand for a negative effect and orange for a positive one). Size of the circles indicates relative sensitivity of each variable in the case of NPRM derived models. Bars represent xR^2 and adjusted R^2 of NPMR and GLM models, respectively.

trait. Temperature variables depicting seasonal variation were important predictors of chloroccocoid, resistent cyanolichens, crustose and foliose broad-lobed species. Despite being selected for less functional groups, seasonal temperature variables were those yielding an overall higher relative sensitivity in the models (Fig. 4).

Precipitation was only selected as a predictor of four lichen functional groups response, and with much lower sensitivity in the models than temperature. These variables were important for almost all type of photobiont functional groups (green lichens were the exception) and for one growth form (squamulose). Seasonal precipitation variables were, as in the case of temperature, those selected as predictors.

Relative humidity was selected only for two growth form models, fruticose and foliose narrow-lobed, but even though with a slightly higher sum of sensitivities than precipitation. On the other hand, aridity was not selected as predictor in any of the models.

discussion

This study presents the first comprehensive work on the main climatic filters acting on lichen trait selection. Overall, we found temperature related variables to be the key predictors of lichen traits response in a Mediterranean climatic gradient. Interestingly, lichens response was not related to mean annual temperature, but rather to its variance year round. Furthermore, the residual importance of precipitation on lichen trait filtering was also related with its seasonal variation, rather than its total amount.

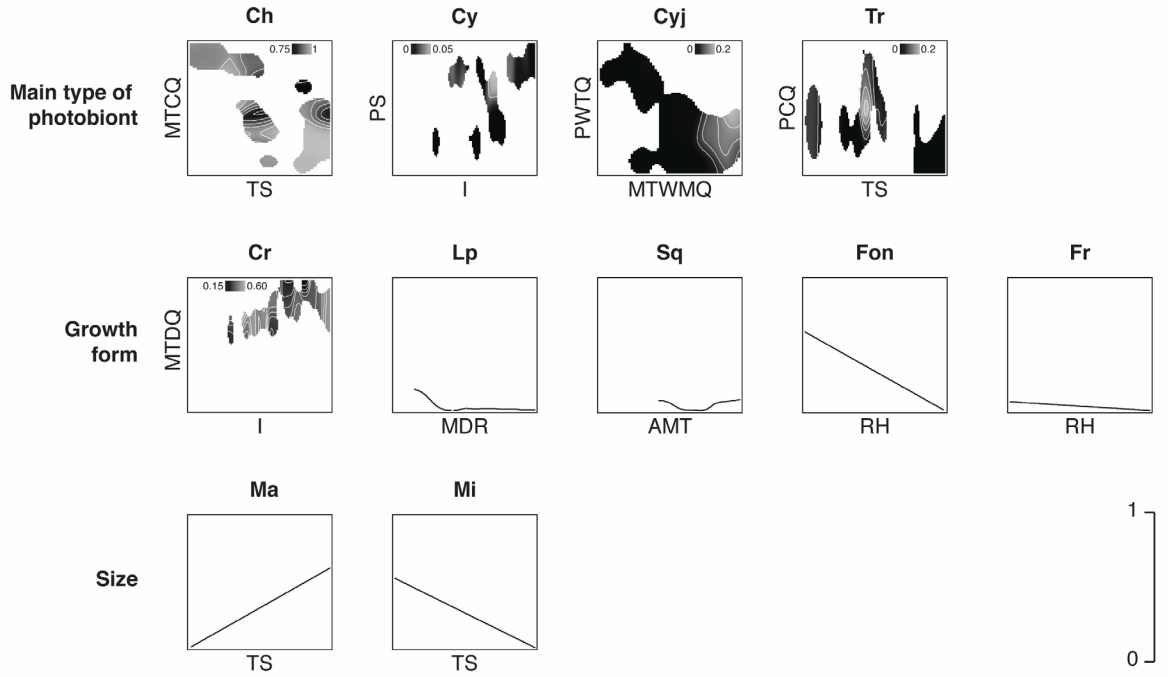

fig. 3. Response of lichen functional groups community weighted mean (CWM) to climate. Surfaces represent the best two-dimensional models and lines represent the best one-dimensional models for each functional group (only models with $R^2 \geq 0.19$ are shown). See table 1 for climate variables decodification and table 2 for functional groups decodification. Models depicted by surfaces or non-linear lines represent non-linear regressions (NPMR) and straight lines represent linear models (GLM).

Lichens are poikilohydric organisms that reflect temporally and spatially the various sources of hydration (Gauslaa 2014). A set of specific trait combinations mediates the relationship with these different water sources (e.g. type of primary photobiont and growth form), placing them among the most responsive to moisture (Gauslaa 2014). Although water is necessary to activate lichens, its physiological activity is highly dependent on temperature. Lichens carbon balance varies with temperature and is also linked to species traits (Sundberg *et al.* 1999; Palmqvist 2000). For instance, different algae or cyanobacteria have their photosynthetic optimum at different temperatures (Bidussi, Gauslaa & Solhaug 2013), so increasing temperatures may lead to photosynthetic inhibition (Hájek, Barták & Dubová 2006), and to a reduction of the photosynthetic periods and increased carbon losses (Shroeter *et al.* 2000). Temperature influences also the speed of hydration and dehydration processes in lichens and other poikilohydric organisms, being linked to species traits such as growth form (de Carvalho *et al.* 2015). Our results highlight temperature's importance, and strengthen its role on trait selection, the mechanism determining community level responses. Temperature had already been identified as the main driver of lichen diversity and trait selection along an altitudinal gradient in Europe (Nascimbene & Marini 2015). The authors conjectured that an enlargement of their temperature gradient to

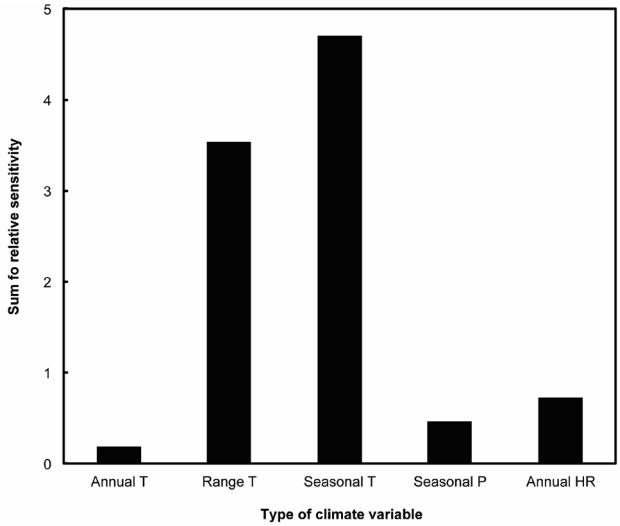

fig. 4. Sum of sensitivity values for each group of climate variables (annual, range and seasonal) selected as predictors of lichen functional groups response, based on linear (GLM) and non-linear regressions (NPMR). Sensitivities were first relativized for each model and averaged by models fit (relative sensitivity x xR^2 or R^2).

more arid conditions would probably result in a stronger effect of precipitation, following the water-energy hypothesis of (Hawkins *et al.* 2003). In fact, water and energy are known drivers of geographical gradients in species richness patterns with evidence suggesting water as the key limiting factor in southern Europe (Whittaker, Nogués-Bravo & Araújo 2007). Although our work is not a continuation of the temperature gradient tested in the Alps, our results suggest that temperature is still the major driver of lichen diversity and trait selection along the vast aridity gradient we encompassed, at least along this gradient. Rather than diminishing the key role of water as a lichen community composition driver and environmental trait filter, we want with our work to highlight the importance of considering likewise temperature. These deepened knowledge on temperature's role on lichen trait filtering is particularly relevant considering the generalized warming trends projected at the global scale (IPCC 2014). The prominent role of temperature on lichen trait selection shows their high sensitivity to temperature and, as already hypothesized (Ellis *et al.* 2007; Aragón, Martínez & García 2012; Nascimbene & Marini 2015), suggests that they may somehow be affected by the forecasted warming trends.

Interestingly, lichen trait response to temperature was not determined by average annual temperature, but rather by its range and seasonal timing. Some works have already demonstrated lichen species sensitivity to seasonal timing and range of temperature variables (Jovan & McCune 2004; Werth, Tømmervik & Elvebakk 2005; Ellis & Coppins 2006; Ellis *et al.* 2007; Ellis & Coppins 2010; Root & McCune 2012), but, to our knowledge, this is the first time that its role on lichen traits filtering is addressed with such detail. Almost all lichen functional groups showed to be highly responsive to temperature range. The importance of temperature range may be related to temperature's

indirect role in atmospheric water-energy dynamics because it determines the availability of water sources (high relative humidity, dewfall, etc.; O'Brien 2006). Large daily temperature ranges determine the existence of dewfall, a recognized source of liquid water for lichens (Gauslaa 2014). Dew formation is more frequent on arid and continental regions, characterized by larger annual temperature ranges and for being less isothermic (the ratio between daily and annual temperature range). These variables were important filters for both cyanolichens functional groups. This is consistent with literature emphasizing dew as a source of liquid water, required for photosynthesis activation in lichens with cyanobacteria as a photobiont (Lange *et al.* 2007; Gauslaa 2014), in continental, arid or even tropical regions (Lange *et al.* 2007; Lakatos *et al.* 2012; Gauslaa 2014). Isothermality was also an important filter of squamulose species, which have a concave growth form that is expected to be more efficient in absorbing dewfall (Vogel 1955). Conversely, in the Mediterranean, low daily temperature ranges occur usually in regions also with lower annual temperature ranges, characterized by being more isothermic and with higher air humidity due to closer proximity to the coast. Consistent with this, lichens with *Trentepohlia* and crustose lichens usually dominate in warm-humid climates (Ellis & Coppins 2006; Marini, Nascimbene & Nimis 2011; Matos *et al.* 2015; Nascimbene & Marini 2015). Lichen functional groups related with type of photobiont were particularly responsive to temperature seasonality and timing. This is consistent with the control temperature exerts on lichen physiology (Sundberg *et al.* 1999). In fact, we found different timings as important filters for different types of algae. This suggests that the control temperature exerts on lichens physiology may be also determining each functional group temperature niche. Resistent cyanolichens are common or even dominant in drylands under increasing aridity conditions (Lange, Belnap & Reichenberger 1998; Belnap, Büdel & Lange 2001; Rogers 2006; Concostrina-Zubiri *et al.* 2014; Giordani *et al.* 2014), which is consistent with their main filter being summer temperatures. On the other hand, the warm-humid conditions required by species with Trentepohlia (Nimis & Tretiach 1995) are in accordance with the main filter being temperature seasonality (its yearly coefficient of variation), which determines warmer conditions year-round. For chlorococcoid lichens both winter temperatures and temperature overall seasonality were selected as main filters. This functional group is able to activate in the absence of liquid water, enabling lichens to live in a wider range of conditions and to have a wider ecological niche (Gauslaa 2014), and these temperature variables may be related to a physiological control.

Precipitation had only a residual importance in our models appearing only in four of them, and mainly in those related to

photobiont type functional groups. This is consistent with water role on lichen photosynthesis activation, which differs between photobiont types (Lange, Kilian & Ziegler 1986). Interestingly, such as in the case of temperature, lichen trait response to precipitation seems to be mediated by its seasonality and timing, rather than by its total amount. The role of seasonal timing of precipitation on lichen community composition was already shown (Ellis *et al.* 2007; Ellis & Coppins 2010; Zedda *et al.* 2011; Root & McCune 2012). However, to our knowledge, this is the first time where seasonal role of precipitation in lichen trait filtering is investigated. The seasonality of rainfall patterns and the amount of winter rain were the determinant precipitation variables. In the Mediterranean, precipitation has a very strong seasonal pattern, being concentrated on the coldest season. Lichens photobiont trait response is in agreement with these patterns of water availability, reflecting water role on lichen photosynthesis activation (Lange, Kilian & Ziegler 1986). These results are very important under a climate change perspective. In fact, summer rainfall addition experiments showed that seasonal changes simulating future climate change scenarios for North American drylands induced changes not only on lichens, but also on mosses (Reed *et al.* 2012; Zelikova *et al.* 2012). On the other hand, an all year round 30% rainfall exclusion experiment in Mediterranean drylands had no effects (Escolar *et al.* 2012). Our work emphasizes the importance of considering the seasonal timing of precipitation, which is particularly relevant under a climate change scenario.

Relative humidity was also an important filter, although to a less extent than temperature or precipitation. Chlorococcoid lichens are able to activate in the presence of humid air, ensuring that relative humidity is around 70% (Lange, Kilian & Ziegler 1986; Nash *et al.* 1990). In our work, foliose narrow and fruticose growth form functional groups, were those related with relative humidity, but with opposite linear relationships. This is consistent with data showing that these narrow-lobed foliose species are more diverse and abundant in dry valleys than in coastal and mountain rainy climates (Gauslaa 2014). Conversely, fruticose species are usually bonded to more humid conditions (Belnap, Büdel & Lange 2001; Giordani *et al.* 2013), being frequenter in oceanic coastal areas (Gauslaa 2014). Although this work was based on an aridity gradient, aridity itself was not selected as the most determinant climate variable for any of the functional groups. This may be primarily related to the fact that aridity is a ratio of precipitation and potential evapotranspiration, and its importance is surpassed in the presence of precipitation and temperature related variables.

Overall, we did not find a common response of functional groups belonging to the same trait to the climatic variables but rather

differential responses to different climatic variables, showing that each trait is limited by different climatic factors. This indicates that functional groups within the same trait occupy different fundamental niches regarding specific climatic requirements. These results are very important taking into consideration climate change predictions. Regarding temperature, southern and central Europe are expected to experience faster summer warming and daytime extremes than mean temperatures, which may partly be ascribed to increases in diurnal temperature range (Kirtman *et al.* 2013). On the other hand, although precipitation projections carry much more uncertainty (IPCC 2014), the fact is that its summer and winter trends from 1950 to 2010 highlight differential seasonal patterns of change, a trend that may continue in the near future (Kirtman *et al.* 2013). Relative humidity, though also with less confidence, is predicted to lower over the 21st century (Collins *et al.* 2013). Under this context, lichen functional diversity differential sensitivity to a broad array of climatic variables, especially those forecasted to change, highlights their potential as a tool to track climate change. Some functional groups exhibited stronger responses than others having a higher potential as ecological indicators of climate change. Functional groups of type of photobiont and size as well as crustose, leprose and foliose growth forms seem particularly promising as ecological indicators to track climate change. Lichens have response to recent climate change as already been demonstrated (Parmesan & Yohe 2003; Aptroot & Van Herk 2007). Our study emphasizes that not only they respond to global warming (Aptroot & Van Herk 2007), but they are also able to potentially signal fine scale changes of seasonal variations of temperature, precipitation and air humidity.

Future research should focus on exploring lichen functional groups potential and developing its use as ecological indicators of climate change. Lichens are ubiquitous and common, existing in a variety of land ecosystems, and with established standard sampling methodologies. Retrospective works may be done crossing the existent studies available since the 80's with climatic information to try to find patterns of change in response to climate change. Lichens were used in the past to track the effects of several sources of global change drivers (from sulphur to metals, and more recently nitrogen). It will be of fundamental importance to disentangle the effect of pollutants, namely those still acting today, from the effects of climate change, and to understand the effects of its interactions. Finally, this work is limited by the quality of the available climatic data. Future work should be done contemplating climatic data with higher spatial continuity and trying to incorporate microclimatic data as a co-variable to improve models' fit and validate these data for other regions.

acknowledgments

This work was supported by FCT-MEC through: project PTDC/AAG-GLO/0045/2014; P.M. by contract SFRH/BD/51419/2011; P.P. by contract SFRH/BPD/75425/2010; A.N. by contract SFRH/BD/51407/2011; C.B. by contract Investigador FCT; P.G. by bilateral scientific cooperation FCT/DREBM 411/00 with CNR Italy; G.A. and I.M. by the Spanish project "EPICON", CGL2010-22049, Ministerio de Ciencia e Innovacion.

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chapter five

Linking shifts in lichen functional groups to the underlying climatic variables: can we do this at a global scale?

publication

Matos P., Pinho P., Giordani P., Aragón G., Martínez I., Nunes A., Soares A.M.V.M., Branquinho C. In preparation.

abstract

Climate change has already impacted natural and human systems worldwide and the scenario will most likely worsen. It is vital to have ecological indicators and biodiversity metrics capable to track and quantify climate change impacts on ecosystems structure and functioning. The use of functional diversity metrics has several advantages. Functional diversity is thought to respond faster and to quantify better ecosystems' response to global change and this response is thought to be more predictable than those of taxonomic diversity metrics, something crucial if we want to use them as an indicator of environmental change. Furthermore, this approach is potentially applicable at the global scale due to its independency from species identity.

A decade ago, lichens response to global warming was observed. More recently, simple lichen traits responding to climate were identified. Further, their detailed response to climate variables with biological relevance was also investigated. Rather than common within the trait, this response to climate was specific of each functional group, and related to the range and seasonal variation of temperature and precipitation, and annual mean relative humidity. This suggests that lichen functional groups have the potential to signal fine scale changes of climate variations. But is it possible to link shifts in lichen functional groups along spatial climate gradients to the underlying climatic variables at a global scale?

To answer this question epiphytic lichen diversity was sampled along a climate gradient covering semi-arid, dry sub-humid, humid and hyper-humid regions in Mediterranean southern Europe. Our results confirm that functional diversity metrics relate with the underlying climate variables in a predictable way. The metrics performed poorly when the entire gradient was analysed simultaneously. We found that the same metric could only be used in homogenous subsets of climatic areas with the same specific limiting climate factors (e.g. seasonal water availability). We found that though the mechanism of lichen functional groups response to climate was transversal to all of them, their use as ecological indicator was only possible in areas with the same limiting factors. Lichen functional groups-based ecological indicators can be applied globally but because they respond to the most limiting climate factors, and these vary as we move in the globe, its application must be done in homogenous areas stratified by specific combinations of limiting factors.

keywords

Mediterranean, lichen response functional groups, photobiont type, growth form, size, ecological indicator of climate

introduction

Monitoring and tracking climate change is a prior concern given the observed and expected worldwide social and ecological impacts. This unprecedented climate change has already impacted natural and human systems worldwide and the scenario will most likely worsen (IPCC, 2014). Although essential to inform us about the state of the environment, monitoring and tracking climate change drivers' is largely insufficient to evaluate climate change impacts on ecosystems. The predicted changes in temperature won't have, for example, the same effect on alpine and desert ecosystems. It is vital to have ecological indicators and biodiversity metrics capable of quantifying and tracking climate change impacts on ecosystems structure and functioning over time (Pereira *et al.* 2013).

Functional diversity metrics can be used to quantify ecosystems response to global change drivers, having several advantages over taxonomic diversity metrics. Functional diversity is thought to respond faster and to quantify better ecosystems' response to global change, providing also a link to ecosystems functioning and their services provision (Díaz & Cabido, 1997, Díaz *et al.* 2007, Lavorel *et al.* 2011, Mouillot *et al.* 2012, Suding *et al.* 2008). This happens because environmental changes rather than immediately altering species richness, may instead induce species shifts; species with traits poorly adapted to these new conditions are removed, and others with specific trait combinations better-adapted to the new environmental conditions are able to re-colonize and replace them (Mouillot *et al.* 2012). This functional approach has the advantage of allowing the identification of these shifts occurring in the community, even if species richness is unresponsive. Another advantage of functional diversity is that these relations between trait-based indices and environmental variables are though to be more consistently monotonic and predictable than those of species richness for instance, something crucial if we want to use them as an indicator of environmental change (Cornwell *et al.* 2006, Mouillot *et al.* 2012). Finally, because this approach is independent of species identity and not constrained by their limited geographical distribution, it has potentially a more universal application.

Lichens responsiveness and sensitivity as ecological indicator of environmental change is widely recognized (Giordani *et al.* 2014, Li *et al.* 2013, Pinho *et al.* 2014, Root *et al.* 2015, Wolseley & Aguirre-Hudson, 1991). In reference to climate, a set of key lichen traits related to the main type of photobiont, growth form and size were identified as responsive (Matos *et al.* 2015, chapter four). Further work revealed that these traits as a whole do not show a common response to climate; the response was instead specific of the functional group (chapter four). This work showed also that the majority of lichen functional groups respond to the range and

seasonal variation of temperature and precipitation, and to a less extent to variations in annual mean relative humidity, suggesting that they have the potential to signal fine scale changes of climate variations. In view of these recent advances and their observed response to global warming (Aptroot & van Herk, 2007, van Herk *et al.* 2002), is it possible to link shifts in lichen functional groups along spatial climate gradients to the underlying climatic variables at a global scale?

Here we try to answer this question with the final aim to develop integrated and global ecological indicators of climate change based on lichen functional groups. Because this functional diversity approach is independent of species identity, we expect each functional group response to be uniquely related to a set of climate variables regardless of the spatial scale of climate, and thus globally applicable. As an example, we expect cyanolichens always respond to precipitation, regardless of the climate or location, and that this functional group can be applied globally to indicate changes in these variables. We expect also that lichen functional groups metrics relate to the underlying climate variables in a predictable way, so they can be used as ecological indicators of climate change. To test these hypotheses, epiphytic lichen diversity was sampled along a climate gradient covering semi-arid, dry sub-humid, humid and hyper-humid regions in Mediterranean southern Europe. This region encompasses several markedly seasonal climates and is expected to be highly impacted by climate change (IPCC, 2014). We expect that the ecological indicators developed here can be applied worldwide.

materials & methods

STUDY AREA

The study area is located in southern Europe across Portugal (n = 64), Spain (n = 25) and Italy (n = 148) (Fig. 1). A total of 237 sites were sampled spanning an aridity gradient based on the Aridity Index (AI) from the United Nations (Middleton & Thomas, 1992) that represents the ratio of mean annual precipitation to mean annual evapotranspiration. Based on it, the UNEP classifies drylands as tropical and temperate areas with an AI < 0.65, sub-dividing it into: hyper-arid (AI < 0.05), arid, (0.05 < AI < 0.20), semi-arid (0.20 < AI < 0.50) and dry–subhumid (0.50 < AI < 0.65). Our sampling included also sites with an aridity index higher than 0.65. Sites with an AI between 0.65 and 0.75 are considered humid, while those with an aridity index higher than 0.75 are classified as hyper-humid regions. Our gradient ranged from Mediterranean semi-arid drylands (0.30 < AI < 0.50) with average minimum and maximum annual temperatures of 4.5ºC and 31.5ºC, respectively, and 522 mm annual precipitation on average, to a hyper-humid region (AI = 1.7) with average

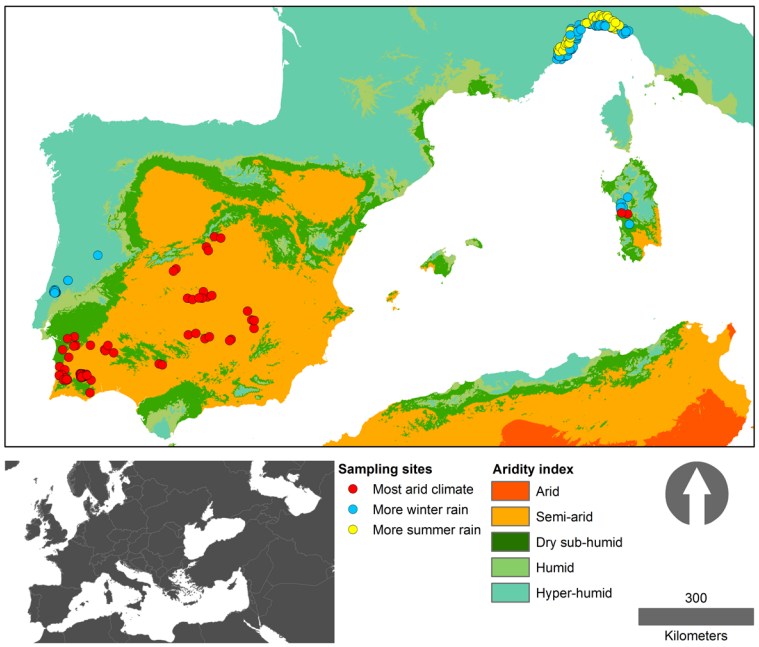

Fig. 1. Map of the study area showing sampling sites and the aridity index (AI) gradient.

minimum and maximum annual temperatures of 1.9ºC and 24.9ºC, respectively, and around 907 mm annual precipitation on average.

LICHEN SAMPLING

Epiphytic lichen communities were sampled in the 237 sites following a standard protocol Asta *et al.* (2002). Due to the large span of climatic conditions, it was impossible to find a common phorophyte along the entire gradient, so 28 different tree species were used. A minimum of 1 and a maximum of 10 trees were sampled in each site, depending on the number of suitable trees found following the indications of Asta *et al.* (2002). A 10 cm x 50 cm grid divided in four 10 cm squares was placed on the four main cardinal directions of the tree trunks, and all species occurring inside the quadrats were registered. The uppermost part of the sampling grid was placed at 1.5 m from the ground and height was adjusted to a maximum of 2 m whenever trunk characteristics were unsuitable for sampling. A total of 299 species were identified (**supplementary table 1**). Species impossible to identify in the field were collected and taken to laboratory for identification.

CLIMATE DATA

For the 237 sites, a set of 21 climatic variables was extracted for the analysis. Nineteen bioclimatic variables were retrieved from WorldClim representing range, seasonal and annual

table 1. Climate variables used in the study.

Main variable	Type		WorldClim code	Variable
Temperature (ºC)	Annual		Bio 1	Annual Mean Temperature (AMT)
	Range		Bio 2	Mean Diurnal Range (MDR)
			Bio 7	Temperature Annual Range (TAR)
			Bio 3	Isothermality (I)
	Seasonal	Seasonality	Bio 4	Temperature Seasonality (TS)
		Summer	Bio 9	Mean Temperature of Driest Quarter (MTDQ)
			Bio 10	Mean Temperature of Warmest Quarter (MTWMQ)
			Bio 5	Maximum Temperature of Warmest Month (MAXT)
		Winter	Bio 8	Mean Temperature of Wettest Quarter (MTWTQ)
			Bio 11	Mean Temperature of Coldest Quarter (MTCQ)
			Bio 6	Minimum Temperature of Coldest Month (MINT)
Precipitation (P)	Annual		Bio 12	Annual Precipitation (AP)
	Seasonal	Seasonality	Bio 15	Precipitation Seasonality (PS)
		Summer	Bio 17	Precipitation of Driest Quarter (PDQ)
			Bio 18	Precipitation of Warmest Quarter (PWMQ)
			Bio 14	Precipitation of Driest Month (PDM)
		Winter	Bio 16	Precipitation of Wettest Quarter (PWTQ)
			Bio 19	Precipitation of Coldest Quarter (PCQ)
			Bio 13	Precipitation of Wettest Month (PWT)
Global aridity index				Annual Mean (AI)
Relative humidity				Annual mean (RH)

variation of temperature and precipitation (see www.worldclim.org for details on variables with codes Bio1 to Bio 19, Table 1). Because sites encompassed also an aridity gradient, the global aridity index was also included in the set (http://www.cgiar-csi.org/data/climate/item/51-global-aridity-and-pet-database). Finally, as lichens respond to other sources of water such as atmospheric moisture (Gauslaa 2014), relative humidity (RH) was also included (extracted from the Biosphere Atlas and built with original data from Climate Research Unit, Univ. of East Anglia, New *et al.*1998).

LICHEN FUNCTIONAL DIVERSITY

This work focused on three lichen response traits and its respective functional groups. Photobiont type, growth form and size (see Table 2 for description of their functional groups)

table 2. Traits and related functional groups (Nimis & Martellos, 2008; Smith *et al.* 2009).

Trait	Functional group	Description	Symbol
Type of primary photobiont	Chlorococcoid	With Chlorococcoid (Green algae)	Ch
	Trentepohlioid	With *Trentepohlia* (Green algae)	Tr
	Cyanolichens	With Cyanobacteria and jelly structure (homoiomerous) or not, that are mostly found in wet climates. See material and methods section for more details	Cy
	Resistant cyanolichens	A specific set of lichens with cyanobacteria and jelly structure (homoiomerous), that are mostly found in dry climates. See material and methods section for more details	Cyj
Growth form	Crustose	Firmly and entirely attached to the substrate by the lower surface	Cr
	Leprose	Like crustose but surface thallus with a granular mass appearance and always decorticated	Lp
	Squamulose	Composed of small scales	Sq
	Foliose narrow-lobed	Partly attached to the substrate with a leaf-like form and narrow lobes	Fon
	Foliose broad-lobed	Same as foliose narrow-lobed but with broad lobes	Fob
	Fruticose	3D-like structure, attached by one point to the substrate with the rest of the thallus standing out from the surface of the substrate	Fr
Size	Microlichens	With crustose or leprose growth form.	Mi
	Macrolichens	With growth form other then crustose and leprose	Ma

were chosen because previous works demonstrated its responsiveness to aridity (Matos *et al.* 2015) and its capacity to respond to temperature, precipitation and relative humidity (chapter four), therefore highlighting its potential to be used as indicators of climate change. Trait classification was retrieved from the Italian database (Nimis & Martellos, 2008) and lichen floras, when absent from that database (Carvalho, 2012, Giralt, 2010, Llop, 2007, Smith *et al.* 2009). The division of regular cyanolichens and resistant cyanolichens was based in species distribution along the gradient (**supplementary Table 1** and **supplementary Fig. 1**). Cyanolichens consistently responding to arid conditions were grouped as resistant cyanolichens. The remaining cyanolichens more bounded to wetter conditions were grouped into cyanolichens.

Species frequencies were calculated as the mean frequency value of each species of all trees samples per sampling site (sites x species frequency). Community level weighted mean (CWM) resulted from the combination of species abundance and trait data (Lavorel *et al.* 2008). Because our traits are categorical, the CWM represents the mean proportion of each functional group in the community, weighted by the abundance of species belonging to those functional groups (Lavorel *et al.* 2008) (CWM of each FG × site). CWM was calculated using the FD package function “dbFD” with CRAN software R (R Core Team, 2013).

STATISTICAL ANALYSIS

Due to the large climate gradient encompassed an analysis was performed on climate variables to ensure a correct classification of the sites into different climates. The definition of different climates was done using a hierarchical agglomerative cluster analysis with Euclidean distance and Ward's method. This analysis was performed on the environmental matrix (sites x climate variables) to aggregate plots into relatively homogenous climate groups based on differences in climate variables. The choice of optimum number of groups to prune the dendrogram was done using Indicator Species Analysis (ISA; Dufrêne & Legendre, 1997). Plots were clustered into up to 17 groups and an ISA analysis was performed on the climate matrix for each group memberships. Resulting *p*-values for each climate variable were averaged for each level of grouping and the number of significant ($p < 0.05$) indicator climate variables was registered. The optimal number of groups was chosen considering a compromise between the lowest *p*-value with the highest number of indicator climate variables (McCune *et al.* 2002). Significance of the groups formed was assed using multi-response permutation procedure (MRPP), with groups considered significantly different if $p < 0.05$. After selecting the optimal number of groups, results from the ISA analysis for that number of groups were used to determine how strongly each climate variable was associated with each group defined to characterize it. Both analysis were adapted from what is commonly done for species composition, but using distance and linkage methods suitable for environmental data (McCune *et al.* 2002). Prior to the analysis, climate data were transformed. A constant was added to eliminate negative values that cannot be handled by Euclidean distances, and data was afterwards relativized by maximum (column) to equally scale climate variables. These analyses were conducted using PC-ORD software version 6.08.

To detect if the prominent gradients driving species composition were related to climate, a non-metric multidimensional scaling (NMS) ordination was performed on the species frequency matrix. Prior to the analysis, data was relativized (by row) to minimize local site characteristics unrelated to the environmental gradient of interest (Matos *et al.* 2015). A Bray–Curtis distance measure was applied due to its good effectiveness on community data (McCune *et al.* 2002). Data underwent 500 iterations per run and the best solution (lowest stress) from 500 runs with real data was chosen, each run beginning with a random configuration. Results strength was evaluated by a Monte Carlo test, relative to 250 runs with randomized data. These analyses were done using PC-ORD software version 6.08. To determine whether climate was underlying the main gradient driving species composition,

the ordinations site scores were correlated with the climate variables. Spearman correlation coefficients were used to account for possible non-linearity (correlations were considered significant for $p < 0.05$). These analyses were performed for the whole dataset, and also separately for each climate using CRAN software R (R Core Team, 2013).

To select the best functional diversity ecological indicators, the response of the CWM of lichen functional groups to climate was modelled. CWM and climate variables were firstly correlated and the linearity of the relationships was visually explored using CRAN software R (R Core Team, 2013). For functional groups showing non-linear responses to climate, a non-parametric multiplicative regression (NPMR) was applied (McCune, 2006). This method accounts both with linear and non-linear relationships between response and predictor variables and with multi factor responses due to interaction between predictors. A local mean estimator and a Gaussian weighting function in a forward stepwise regression were used, optimizing at the same time the smoothing parameters (tolerance). Minimum average neighbour size was set to three. Leave-one-out-cross-validation was used to control over-fitting (medium over-fitting was selected) and a cross-validated R^2 (xR^2) was calculated as a measure of fit. The relative importance of individual explanatory variables within the selected models was evaluated by a sensitivity analysis (McCune 2006). A sensitivity value of zero indicates that the predictor has no detectable effect on the response, while a sensitivity value of one means that on average nudging that predictor results in a response change of equal magnitude. For functional groups showing linear relationships with climate variables, least squares multiple linear regressions were used (MLR). These models were chosen over, for instance, generalized linear models (GLM) to enable quicker comparisons with NPMR models. As in NPMR, an xR^2 was calculated using a leave-one-out strategy (in addition to the traditional R^2). Models were run individually for each functional group and climate variable. In the case of NPMR all climate variables combination were also tested and the selection of best models followed a rule of maximum xR^2 with the smallest number of predictors. Models with two or more predictors were kept only if its xR^2 increased by more than 5% over the simplest one. A randomization test was used to assess all models significance, comparing the estimation of selected models with an average estimation calculated by 250 random permutations among the dataset. These analyses were also run for the whole dataset, and separately for each climate dataset that was previously shown to be a main driver of species composition. Models were run in Hyperniche 2.27 software.

In a simple and consensual way, a good ecological indicator or

surrogate is one that can be feasibly measured in a cost-effective way, that can be representative of the process of interest and that responds promptly and consistently in a predictable way (Niemeijer & de Groot, 2008). For this reason, our selection of the indicators was based not only on the significance of the modelled relationships with the climate variables and its fit, but also in the shape of those relationships to assure a straightforward predictability. Functional groups with non-monotonic relations with climate were excluded from the set of ecological indicators of climate.

results

CLIMATE CHARACTERIZATION

Climate similarity between sites was investigated using hierarchical agglomerative cluster analysis. The cluster dendrogram was prune into three groups, as this level of grouping yielded the best combination of highest indicator climate variables with the lowest *p*-value (following (Dufrêne & Legendre, 1997). Final plot assignment into groups can be found in **supplementary Table 2** and its spatial location in Fig. 1. These three groups aggregated homogenous sites in terms of

table 3. Best climate variable indicators of each climate obtained with a Indicator Species Analysis (ISA). Significant indicator values are shown in bold.

Climate classification	Climate variable			Code	Indicator value
More Arid	Temperature	Annual		AMT	**37.4**
		Range		MDR	**37.6**
				I	**34.9**
				TAR	**35.5**
		Seasonality	Summer	MTWMQ	**36.5**
				MTQC	**37.0**
				MAXT	**37.6**
			Winter	MTWTQ	34.4
				MTQC	**39.7**
				MINT	**40.0**
	Precipitation	Seasonality		PS	**37.1**
More Winter Rain			Winter	PWTM	**36.0**
				PWTQ	**36.7**
				PCQ	**36.9**
More Summer Rain	Temperature	Seasonality		TS	**35.2**
	Precipitation	Annual		AP	**39.4**
		Seasonality	Summer	PDM	**38.9**
				PDQ	**44.7**
				PWMQ	**44.3**
	Aridity	Annual		AI	**47.6**
	Relative Humidity	Annual		RH	**33.8**

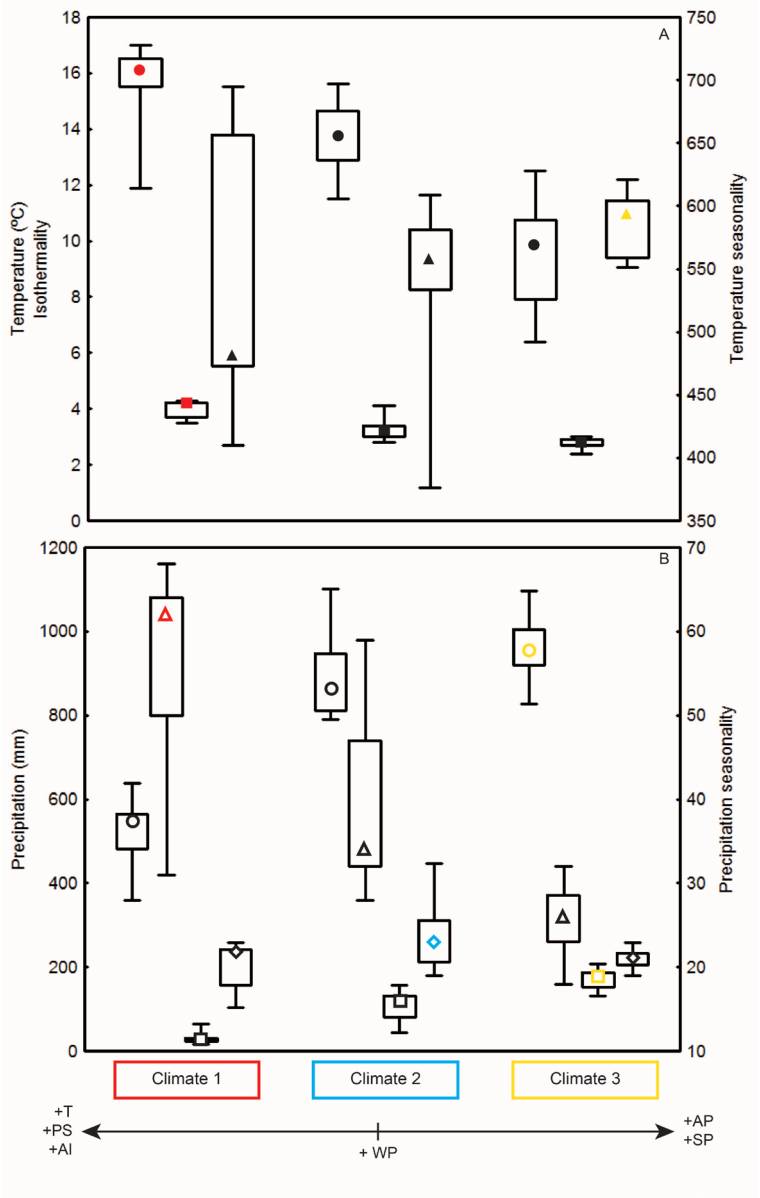

fig. 2. Climate variation in the study area and its characterization. Temperature (A) and precipitation (B) descriptive statistics for each climate classified based on climate similarity between sites. The most differentiating variables of each climate classification are highlighted with that climate classification color: red - the most arid climate; blue - the climate with more winter rain; yellow - the climate with more summer rain. Solid symbols: circle - annual mean temperature; square - isothermality; triangle - temperature seasonality. Open symbols: circle - annual precipitation; square - summer precipitation; triangle - precipitation seasonality; diamond - winter precipitation. Boxes indicate 25% - 75% quartiles, closed circles indicate median and whiskers indicate non-outliers minimum and maximum.

climate, significantly different from each other ($p < 0.001$). The ISA analysis allowed the determination of how strongly each climate variable was associated with each group defined (Table 3). The climatic characterization of each group is summarized in Fig. 2. Overall higher annual, summer and winter temperatures characterize the first climate, hereafter called as the most arid, which included 73 sites. Sites belonging to this climate have also, higher precipitation seasonality. The second group aggregates 100 sites showing the highest winter precipitation, hereafter called as the climate with more winter rain. Finally, the third group, hereafter called as the climate with more summer rain, is the less arid and it's characterized by overall higher annual and summer precipitation. This climate clusters 64 sites.

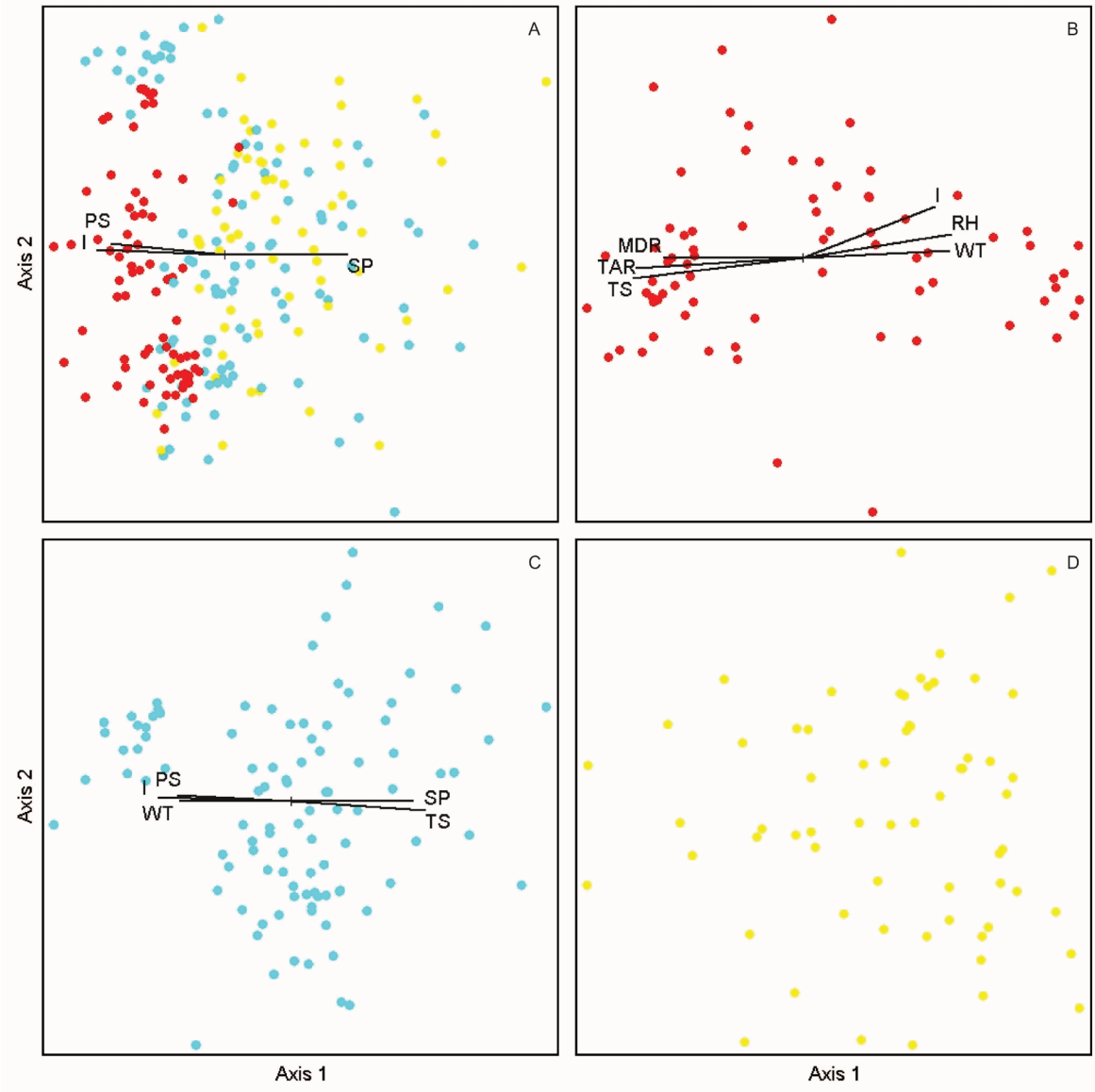

fig. 3. Non-metric multidimensional scaling analysis (NMS) of sampling sites according to lichen communities for the whole dataset (A), for the most arid climate (B), for the climate with more winter rain (C) and for the climate with more summer rain (D). Vectors represent significant correlations between the first axis and climate variables. To prevent overcrowding, only the highest correlation and those falling within a 5% margin of the highest are shown. Sites are color-coded by type of climate classification: red - the most arid climate; blue - the climate with more winter rain; yellow - the climate with more summer rain.

LICHENS COMMUNITY RESPONSE TO CLIMATE

Sites were ordinated based on lichen species composition and color-coded using the three climate groups (Fig. 3, A). The ordination had three dimensions, as the addition of a fourth one only slightly reduced minimum stress. Final stress was 15.9%, with minimum stress lower than would be expected by chance ($p = 0.004$). The NMS ordination explained 58.7 % of the community variation, distributed mostly between the first and second axes (25.3 % and 21.2 %, respectively), while the third one represented only 12.2 % of the variation in lichen species communities. The main gradient driving species composition

table 4. Summary of Spearman correlations (ρ) between non-metric multidimensional scaling ordination axis 1 and climate variables. Correlations are shown for ordinations of the whole dataset (N = 237), for the most arid climate (N = 73), for the climate with more winter rain (N = 100) and for the climate with more summer rain (N = 64).

Climate variable			Code	Whole gradient		Most arid climate		More winter rain		More summer rain	
				r^2	p	r^2	p	r^2	p	r^2	p
Temperature	Annual		AMT	-0.72	<0.001	0.56	<0.001	-0.49	<0.001	0.16	0.216
	Range		MDR	-0.67	<0.001	-0.8	<0.001	-0.4	<0.001	0.05	0.692
			I	0.04	0.502	-0.82	<0.001	0.48	<0.001	-0.14	0.281
			TAR	-0.8	<0.001	0.78	<0.001	-0.59	<0.001	0.17	0.185
	Seasonality	Summer	MTWMQ	0.49	<0.001	-0.83	<0.001	0.58	<0.001	-0.18	0.161
			MTQC	-0.72	<0.001	0.8	<0.001	-0.57	<0.001	0.18	0.165
			MAXT	-0.6	<0.001	-0.52	<0.001	0.09	0.375	0.14	0.254
		Winter	MTWTQ	-0.62	<0.001	-0.69	<0.001	-0.05	0.603	0.1	0.423
			MTQC	-0.15	0.017	0.47	<0.001	0.22	0.031	-0.08	0.506
			MINT	-0.57	<0.001	-0.6	<0.001	0.14	0.154	0.16	0.211
Precipitation	Seasonality		PS	-0.67	<0.001	0.81	<0.001	-0.59	<0.001	0.21	0.102
		Winter	PWTM	0.62	<0.001	0.43	<0.001	0.13	0.21	-0.18	0.152
			PWTQ	0.5	<0.001	0.53	<0.001	-0.05	0.638	-0.16	0.208
			PCQ	0.78	<0.001	-0.63	<0.001	0.64	<0.001	-0.18	0.163
Temperature	Seasonality		TS	-0.74	<0.001	0.75	<0.001	-0.54	<0.001	0.02	0.885
Precipitation	Annual		AP	0.78	<0.001	-0.68	<0.001	0.62	<0.001	-0.25	0.049
	Seasonality	Summer	PDM	0.45	<0.001	0.59	<0.001	-0.21	0.034	-0.18	0.164
			PDQ	-0.18	0.006	0.62	<0.001	-0.43	<0.001	0.01	0.952
			PWMQ	0.78	<0.001	-0.61	<0.001	0.6	<0.001	-0.17	0.172
Aridity	Annual		AI	0.71	<0.001	0.68	<0.001	0.49	<0.001	-0.22	0.074
Relative Humidity	Annual		RH	0.52	<0.001	0.8	<0.001	0.38	<0.001	-0.16	0.203

was highly significantly correlated with climate variables (Table 4). This main axis of lichen community composition reflects a climate gradient ranging from sites with higher isothermality and precipitation seasonality, to sites with higher summer precipitation (Fig. 3 A). This axis clearly separates sites from the most arid climate, from those with more winter and more summer rain, but not between the latter two.

The ordinations of lichen community composition taken separately for each climate had three dimensions, as adding another one did not significantly reduced minimum stress. Final stress for each of ordination was 11.1 %, 14 % and 16.4 %, respectively, all lower than would be expected by chance (p = 0.004). The three ordinations accounted on total for 81.6 %, 64.8 % and 59.2 %, respectively, of the variation in the community. Correlations between the first axis of these ordinations and the climates variables are summarized in Table 4. The lichen community variation in the most arid climate was mostly distributed in the first axis (53 % in axis 1, 17.2 % in axis 2 and 11.4 % in axis 3). This axis represents a gradient from sites with high annual and daily temperature ranges and seasonality, to

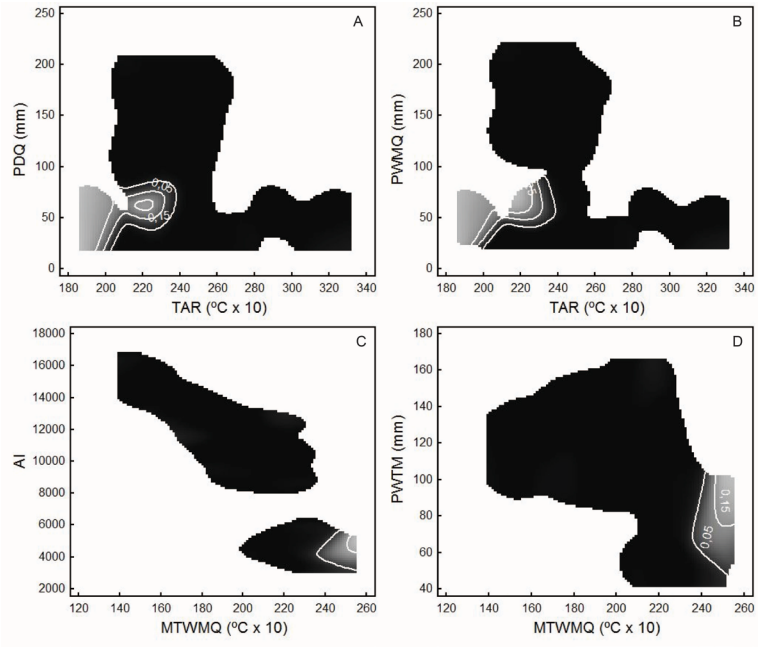

fig. 4. Response surfaces of lichen functional groups community weighted mean (CWM) to climate along the entire climate gradient. Surfaces represent the best two-dimensional non-parametric multiple regression (non-linear) models. A and B - cyanolichens; C and D - resistant cyanolichens. See table 1 for climate variables decodification.

sites with high isothermality, minimum temperatures and relative humidity. The first axis of lichen community composition in the climate with more winter rain accounted also with the highest variation (31 % in axis 1, 21.8 % in axis 2 and 12 % in axis 3), and followed a gradient from sites with high minimum temperatures, precipitation seasonality and isothermality, to sites with high summer precipitation and temperature seasonality. The lichen community composition of the climate with more summer rain was likewise mainly distributed on the first axis (31.6 % in axis 1, 18.9 % in axis 2 and 8.7 % in axis 3), but the gradient was only marginally correlated with summer precipitation (R^2 = -0.25, p = 0.048). Because the main driver of species composition of this subset was not climate, this subset was discarded from further analysis.

FUNCTIONAL GROUPS RESPONSE TO CLIMATE

All functional groups were significantly predicted by at least one climate variable (**supplementary Table 3**). From this set of 12 functional groups with significant models, eight were selected as ecological indicators of spatial changes in climate due to their consistent monotonic responses along the climate gradients (Fig. 4 to 7). Best models for each functional group and its descriptions are shown in Table 5. Functional groups belonging to the same trait showed differential responses to climate in terms of predictors and response strength (Fig. 8). It was evident from results (Fig. 8) that some functional groups response was climate specific. While some were exclusive to a certain climate, others

table 5. Best climate predictor models for lichen functional groups (FG) response. See table 1 for climate variables names and symbols, and table 2 for functional group names. Models are shown for the whole dataset (Total) and for each climate (Clim.1 - the most arid climate, Clim. 2 - the climate with more winter rain).

						NPMR							MLR	
Trait	FG	Data	Var. 1	Var. 2	No.	xR^2	p	Avg.	Tol. 1	Tol. 2	Sen. 1	Sen. 2	Const.	Coef.
Photobiont	Cy	Total	TAR	PWMQ	2	0.63	0.004	17.8	7.3	15.28	0.19	0.10		
			TAR	PDQ	2	0.62	0.004	16.4	7.3	12.12	0.20	0.10		
		Clim. 1	I	PWTM	2	0.77	0.004	15.6	0.72	3.7	0.26	0.27		
			I	PWTQ	2	0.73	0.004	15.1	0.48	10.72	0.21	0.21		
		Clim. 2	I		1	0.62	0.004						0.54	0.000
			TS		1	0.58	0.004						-0.49	0.016
	Cyr	Total	MTWMQ	PWTM	2	0.64	0.004	16.3	5.8	10	0.42	0.16		
			MTWMQ	AI	2	0.60	0.004	15	8.12	414.5	0.34	0.40		
		Clim. 1	MTWMQ	PWTM	2	0.77	0.004	18.8	5.39	8.71	0.38	0.13		
			MTWMQ	PS	2	0.77	0.004	14.4	3.43	5.18	0.50	0.09		
			MTWMQ	AI	2	0.75	0.004	17.9	6.37	337.9	0.41	0.29		
			MTWMQ	PWTQ	2	0.73	0.004	15.2	3.43	28.62	0.47	0.07		
	Tr	Clim. 1	RH		1	0.26	0.004						-0.47	0.008
			MINT		1	0.22	0.004						0.21	-0.001
			TS		1	0.22	0.004						0.18	0.000
Growth form	Cr	Clim. 1	RH		1	0.46	0.004						-1.51	0.028
			TS		1	0.43	0.004						0.95	0.000
			TAR		1	0.41	0.004						1.05	-0.002
			MAXT		1	0.41	0.004						2.01	-0.005
	Fb	Clim. 2	TS		1	0.44	0.004						1.34	0.000
			I		1	0.43	0.004						-0.93	0.034
	Fn	Clim. 1	TS		1	0.71	0.004						-0.69	0.000
			TAR		1	0.70	0.004						-0.87	0.004
			MINT		1	0.67	0.004						0.64	-0.007
			RH		1	0.66	0.004						3.51	-0.048
	Fr	Clim. 1	TS		1	0.29							0.23	0.000
			TAR		1	0.28							0.27	-0.001
			RH		1	0.27							-0.54	0.009
			MINT		1	0.25							-0.01	0.001
Size	Ma	Clim. 1	TS		1	0.70	0.004						-0.32	0.000
			RH		1	0.69	0.004						3.30	-0.042
			TAR		1	0.65	0.004						-0.46	0.004
	Mi	Clim. 1	TS		1	0.70	0.004						0.00	1.325
			RH		1	0.69	0.004						0.04	-2.298
			TAR		1	0.65	0.004						0.00	1.457

were indicators of more than one, but the climate variables they respond to are specific of each climate.

In reference to the whole gradient, only two functional groups of main type of photobiont were selected. Both functional groups responded to temperature and water related variables

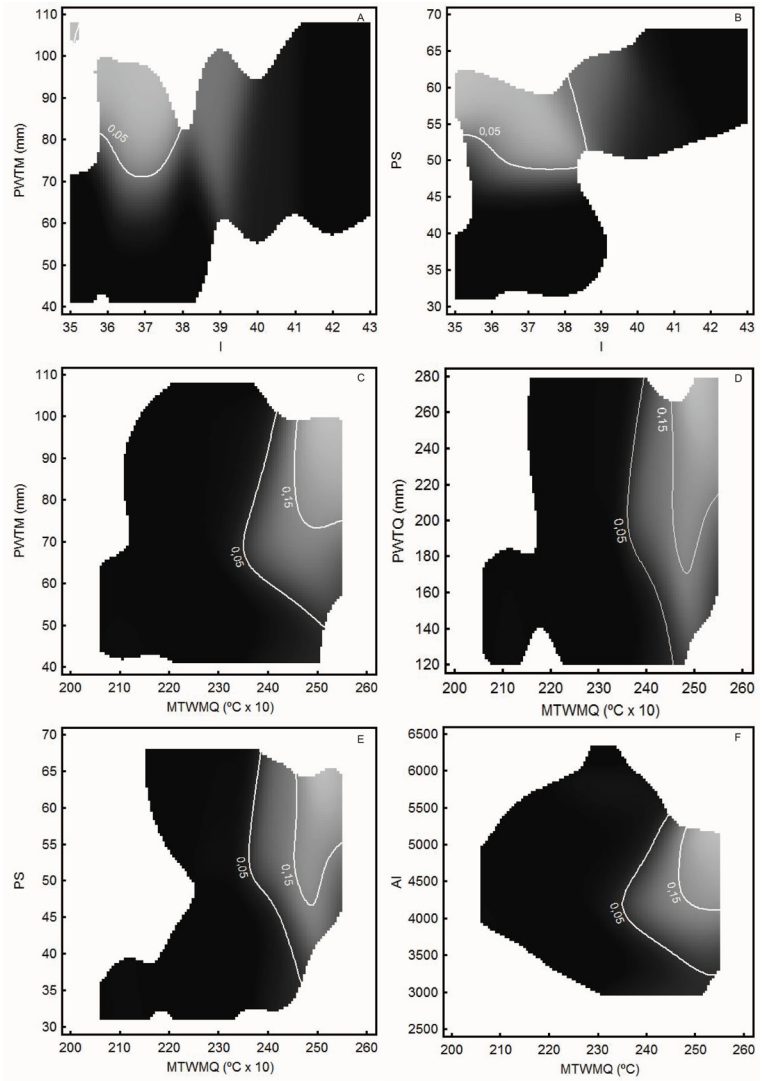

fig. 5. Response surfaces of lichen functional groups community weighted mean (CWM) to climate along the most arid climate. Surfaces represent the best two-dimensional non-parametric multiple regressions (non-linear) models. A and B - cyanolichens; C to F - resistant cyanolichens. See table 1 for climate variables decodification.

(precipitation and aridity, which is calculated using precipitation). Cyanolichens and resistant cyanolichens showed the most significant and consistent response along this gradient (Fig. 4). Both had two-dimensional non-linear models (Table 5). Cyanolichens CWM decreased consistently with increasing temperature range (TAR) and precipitation seasonality in this gradient (Fig. 5 A and B). On the other hand, resistant cyanolichens CWM increased consistently with increasing summer temperatures (MTWMQ) and aridity (low values of the aridity index refer to increasing values of aridity) occurring simultaneously with decreasing amounts of winter precipitation (PWTM, Fig. 5 C and D).

Eight functional groups from all traits responded consistently to climate along the gradient in the most arid climate. Like in the entire gradient, all functional groups responded to water related variables and temperature. Both cyanolichens functional groups showed bi-dimensional non-linear responses to climate (Fig. 5).

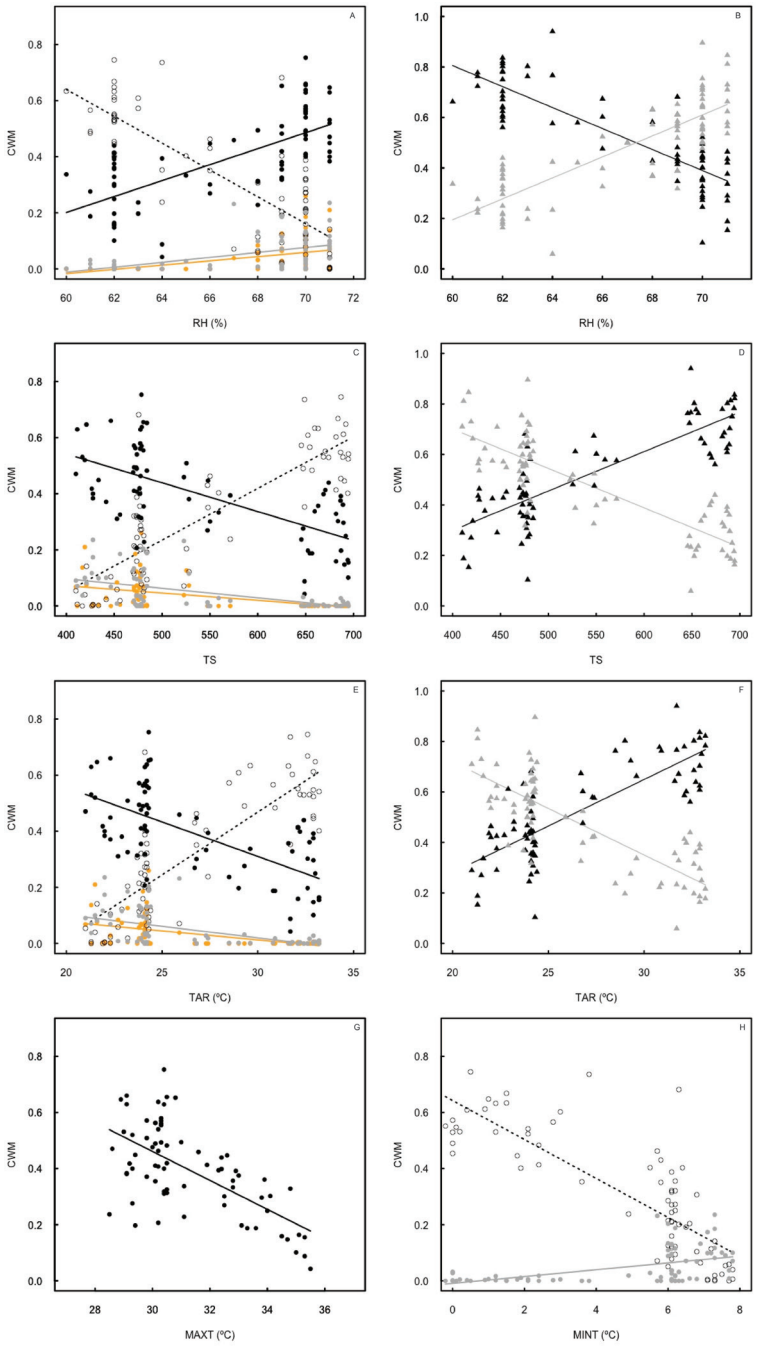

fig. 6. Response of lichen functional groups community weighted mean (CWM) to relative humidity (RH), temperature seasonality (TS), annual range (TAR) and maximum (MAXT) and minimum (MINT) temperatures. Lines represent the best one-dimensional least-squares multiple linear regression models for the most arid climate. *Trentepohlia* - solid orange circles and line; Crustose - solid black circles and line; Foliose narrow lobed - hollow black circles and dashed line; Fruticose - solid grey circles and line; Squamulose - hollow red circles and solid line; Macroclichens - solid line and black triangles; Microlichens - solid line and grey triangles. See table 1 for climate variables decodification.

In this climate, cyanolichens CWM increased with increasing winter precipitation (PWTM) and precipitation seasonality if these occurred together with decreasing levels of isothermality (Fig. 5 A and B). Resistant cyanolichens, showed the same response to winter precipitation (PWTM and PWTQ) and precipitation seasonality as in the entire gradient, but only if coinciding with increasing high summer temperatures (MTWMQ, Figs. 5 C to E). Resistant cyanolichens responded also to aridity under this climate gradient but not so clearly (Fig. 5 F). The other six functional groups responded linearly to climate variables along the

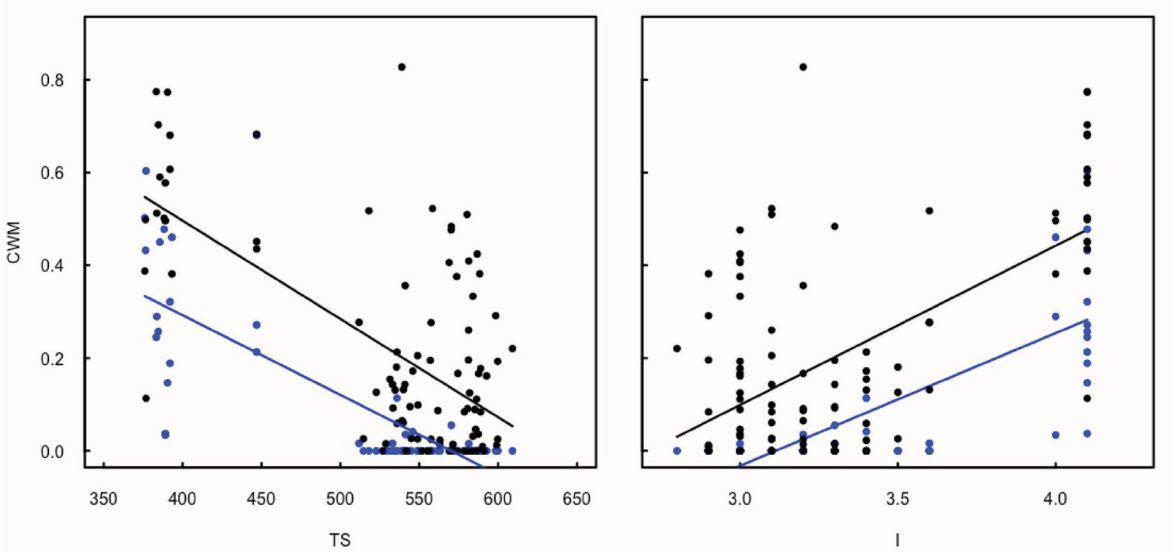

fig. 7. Response of lichen functional groups community weighted mean (CWM) to temperature seasonality (TS) and isothermality (I). Lines represent the best one-dimensional least-squares multiple linear regression models for the climate with more winter rain. Cyanolichens - solid blue circles and line; Crustose - solid black circles and line. See table 1 for climate variables decodification.

gradient (Fig 6). The results showed clear shifts in the CWM of the dominant functional groups of growth form. Foliose narrow lobed species shifted their relative dominance with crustose species as relative humidity increased, and to a lesser extent also fruticose species (Fig. 6 A). Species with *Trentepohlia* showed also the same response as fruticose and crustose species, increasing their relative abundance as relative humidity increased (Fig. 6 A). Shifts in the relative dominance were also obvious in reference to lichen size (Fig. 6 B). Macrolichens dominated in sites with the lowest relative humidity, and as this increased microlichens became dominant. Similar patterns and shifts were observed for temperature seasonality (Fig. 6 B and C) and temperature annual range (Fig. 6 D and E), but with the opposite trend. Crustose species showed also a consistent decrease with increasing maximum temperatures. Finally, in response to an increase in minimum temperatures, foliose narrow species decreased its relative abundance while fruticose species increased.

In the climate with more winter rain only two functional groups responded with linear one-dimensional models (Fig. 7). In this climate, lichen functional groups response was not related to water, but exclusively to temperature. Both cyanolichens and crustose species decreased in this climate with increasing temperature seasonality and increased with isothermality.

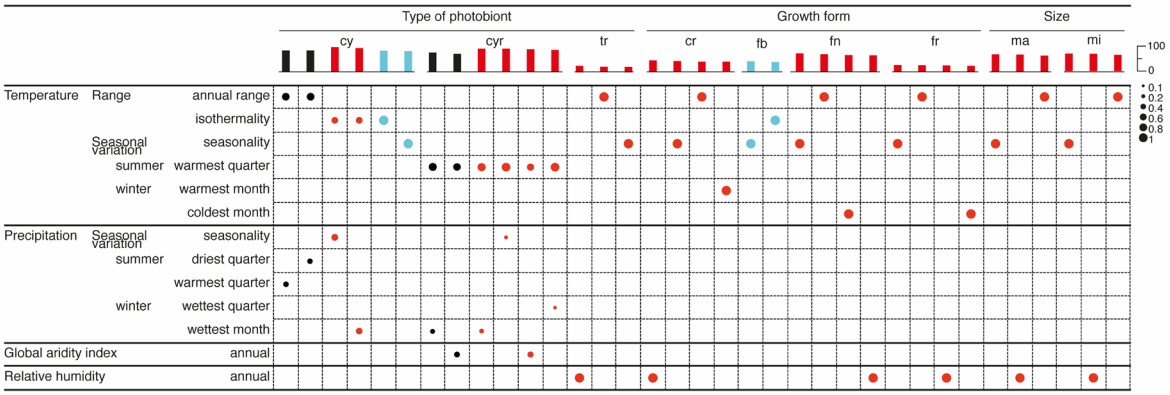

fig. 8. Best climate predictors for the CWM of each functional group model selected as ecological indicator of spatial changes in climate. Uni-dimensional models represent linear regressions (MLR) and bi-dimensional models represent non-linear regressions (NPMR). Models done for the entire dataset are represented in black, while red and blue models stand for the most arid climate and the climate with more winter rain, respectively. Size of the circles indicates relative sensitivity of each variable in the model. In one-dimensional models sensitivity is 1. Bars represent xR^2.

discussion

As hypothesized, lichen trait-based metrics showed consistent predictable monotonic responses to climate. Based on them, we identified for the first time functional groups-based lichen ecological indicators of climate change for different Mediterranean climate areas delimited by different limiting climate factors. Our results showed that lichen communities and functional group-based metrics respond to the most limiting climate factor, and this mechanism is transversal to all functional groups. However, because the most limiting factors vary spatially, the use of their metrics is restricted to specific areas with the same combination of limiting factors. Thus, unlike expected, the same functional group metrics cannot be applied at a global scale. Nonetheless, our results indicate that the methodology has the potential to be globally applicable, as long as its application is made in homogenous areas stratified by similar combinations of limiting climate factors.

MAIN CLIMATE GRADIENTS ACTING AS ENVIRONMENTAL CLIMATE FILTERS ON LICHEN COMMUNITIES' ASSEMBLY

After realizing that lichen functional groups did not respond as expected to the whole large-scale climate gradient encompassed in this work, we decided to divide the study area into smaller and homogenous climate areas. A cluster analysis of the climate variables in the study area helped classify the study area into three different main climates: i) on one extreme we have sites with higher overall temperature and low amounts of very seasonal precipitation, mostly concentrated on winter; ii) in the middle of the gradient a climate with milder temperatures and more winter precipitation but less markedly seasonal; iii) and on the other extreme of the gradient, a climate with more temperate temperatures with higher precipitation amounts and

more evenly distributed between winter and summer. Basically the whole spatial climate gradient can be seen as a temperature-precipitation amount and seasonality axis (Fig. 2).

The analysis of lichen communities along the entire gradient and the three sub climates revealed that they respond directly to the main climate limiting factors. In the entire gradient, the spatial temperature-precipitation amount and seasonality operate as primary filters in lichen species composition along this gradient. This is in accordance with the ecological hypothesis that environmental drivers work as hierarchical filters restraining the assemblage of communities, selecting locally for the best adapted species available from the regional pool (Bello *et al.* 2013, Weiher & Keddy, 1995, Woodward & Diament, 1991). Taken separately by climate, temperature and precipitation acted also as environmental filters determining lichen communities assemblages in the most arid climate and in the climate with more winter rain. However, in the climate with less seasonal but higher amounts of precipitation climate was not the main environmental filter. This climate aggregated sites with a smaller climate variation regarding the climate variables used. Under these circumstances other small scale drivers like radiation, microclimate or biotic interactions may act predominantly (Bello *et al.* 2013). Future works should try to unfold these relationships with small-scale drivers, and to understand to which extent can such responses affect lichen functional groups performance as macroclimate indicators. Thus we do not discuss it further.

LICHEN FUNCTIONAL GROUPS RESPONSE VARIES WITH LIMITING FACTORS

Lichen trait-based metrics responded directly to the limiting factors along the entire gradient and in each of the sub climates. All traits and respective functional groups showed this behaviour indicating that the mechanism of response is transversal to all of them. However, because these limiting factors vary along space with climate, functional group-based metrics varied with it (Fig. 9). Thus, unlike expected, each lichen functional group response is not unique but rather specific to the limiting factor, varying as we move to areas with different combinations of limiting factors.

A decade ago, an impressive work alerted for the importance of assessing the global contribution of climate controls on global vegetation, as a means to more comprehensively understand the effects of climate change (Nemani *et al.* 2003). Because temperature, water and radiation interact and set complex and varied limits on vegetation activity (Churkina & Running, 1998), the authors estimated the percentage of earth vegetated surface limited by these co-limiting factors (Nemani *et al.* 2003). Basically

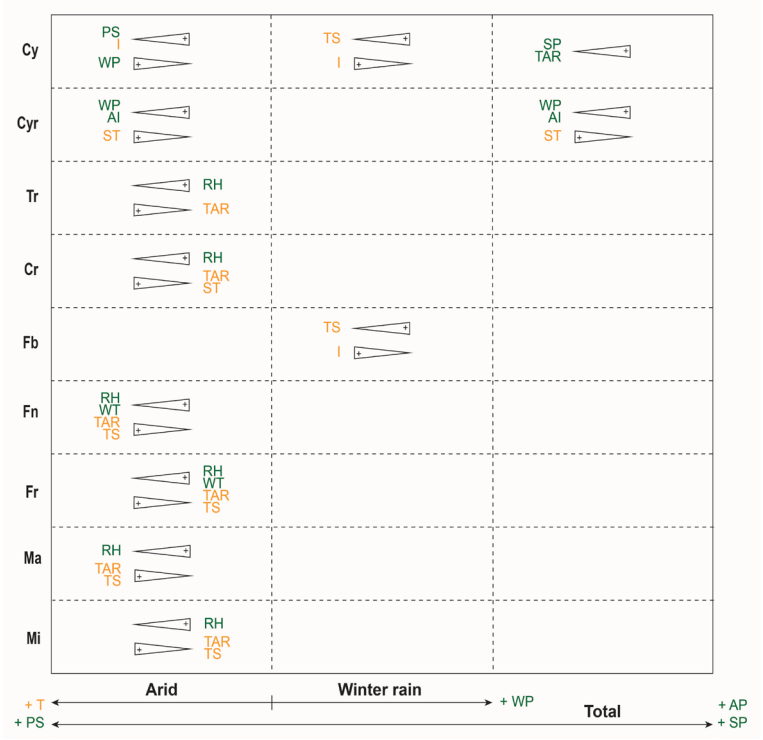

fig. 9. Diagram of best lichen-based functional diversity ecological indicators of spatial changes in climate. Best indicators are shown for each climate and for the entire gradient, with indication of climate variables. Climate variables are represented by triangles illustrating a gradient from lower values (vertex) to higher values. Codes for climate variables are placed on the side of the gradient where the functional group is more abundant. Water related variables are coloured in green and temperature related variables in orange. Aridity is coloured in black. The main climatic drivers underlying each gradient are also shown in diagram. See table 1 for climate variables names and symbols, and table 2 for functional group names.

as we move on earth, the percentage of each of these factors changes, changing the most limiting factor. For this reason, while in the most arid climate where water is a limiting factor cyanolichens respond to precipitation, in the climate with more winter rain they only respond to temperature variables as water is no longer a limiting factor (Fig. 9). Our findings are of crucial importance, because they show how lichens integrate and illustrate this complex and diverse control imposed by climate on vegetation activity. Rather than a setback on its global application, this shows that because their response is conditioned to the limiting factor, and these vary over space, each time we move their response should be reinterpreted alight of a new combination of limiting factors, and the adequacy of the indicator to this new combination of limiting factors should be considered.

The predominance of temperature variables linked to the functional structure of lichen communities along the spatial climate gradients, namely its seasonal variance and range, is consistent with recent findings (chapter four). Lichens are poikilothermic organisms, meaning that they respond and depend directly on the surrounding environment temperature to regulate their physiological activity. Temperature is known, for instance, to affect both respiration rates (Kershaw, 1985, Nash III, 1996) and photosynthesis (Bidussi *et al.* 2013, Gauslaa & Solhaug, 1999), together with water and light. For this reason, it is not surprising that temperature variables assume such great relevance as an environmental filter of lichen functional traits.

Unlike temperature, lichen functional groups response to water occurred only when water was one of the limiting factors. Water and energy are known drivers of geographical gradients in species richness patterns with evidence suggesting water as the key limiting factor for a number of organisms in southern Europe (Whittaker *et al.* 2007). Our results suggest that with lichens, water is in fact a stronger driver in regions where it is the limiting, but always together with temperature. In accordance with recent findings (chapter four), we also found precipitation seasonal variation, rather than its total amount to be the limiting factor. Interestingly, in the most arid climate, lichen functional groups response to water was not confined to rain, and included also relative humidity. Lichens are known to respond to several different of precipitation, ranging from rain, dew and fog, which become increasingly important in places of low rainfall inputs (Gauslaa, 2014). Our results show that they can be used as ecological indicators of spatial changes in in water, including other forms besides rainfall.

FUNCTIONAL GROUPS-BASED LICHEN METRICS CAN BE APPLIED GLOBALLY

Lichen functional groups-based ecological indicators can be applied globally but because they respond to the most limiting climate factors, and these vary as we move in the globe, its application must be done in homogenous areas stratified by specific combinations of limiting factors. Liebig's law (1840) states that the limiting factor is the one that is least available at a particular time. This has been suggested under an ecological indicator perspective (Hiddink & Kaiser 2005). If an ecological indicator is based on abundance metrics, like these trait-based indicators used in our work, large spatial scales encompassing several climatic limiting factors may pose a problem in the determination of their spatial patterns (Hiddink & Kaiser 2005). In our work, when the entire spatial gradient was considered, several underlying limiting factors acted simultaneously, obscuring traits response (Fig. 9). When the study area was divided by homogenous climates narrowing the number of climate limiting factors, the indicators response improved and more functional groups became responsive. Thus, lichen traits can be applied globally, but the selection and interpretation of the lichen trait-based indicators must be done accordingly, and results must be analysed separately for areas with specific limiting factors.

LICHEN FUNCTIONAL GROUPS AS INDICATORS OF CHANGES IN CLIMATE

Our results corroborate functional ecology hypothesis that

functional diversity metrics relate monotonically with the underlying climate variables in a predictable way (Cornwell, Schwilk & Ackerly 2006; Mouillot *et al.* 2012). Lichen functional groups-based indices (community weighted mean, CWM) responded consistently and monotonically in a predictable way to climate along spatial gradients. Thus they can be used as ecological indicators of climate change in space.

Eight lichen functional groups were identified as good ecological indicators of spatial climate change for the most arid climate (Fig. 9). In reference to relative humidity and temperature seasonality and annual range, we observed a clear shift between the functional groups of growth form. Shifts in lichen functional groups have already been observed in response to nitrogen pollution (Pinho *et al.* 2011). To the best of our knowledge, this is the first time that shifts in functional groups of lichens communities are reported for climate. In this type of arid climate, foliose narrow lobed species will shift their relative dominance, being replaced by crustose and fruticose species with increasing levels of relative humidity. Also the relative abundance of Trentepohlioid species is a good indicator of increasing levels of relative humidity in these regions. The same shifts in the relative abundance of these functional groups were observed in response to temperature seasonality and annual range, but with the opposite trend. Size, an even simpler measurable trait, can also be used to track changes in these climate variables in these regions. A shift from macro to microlichens dominated communities can be used to indicate increasing levels of relative humidity or decreasing temperature seasonality and annual range in this arid climate. Decreasing levels in the relative abundance of crustose species can also be used to track an increase in the maximum temperature over this gradient. On the other hand, to track an increase in minimum temperature both increases in the relative abundance of fruticose, or decreases in the relative abundance of foliose narrow can be used. Cyanolichens occur in relative low abundances in this climate, and under specific conditions of low isothermality, and high winter precipitation and precipitation seasonality. The relative abundance of this group in this climate can be used to track decreases in winter precipitation or its seasonality, and to track changes in isothermality. Resistant cyanolichens can be used in these regions as ecological indicators to track summer temperature or aridity, as in this climate they can be increasingly found as we move towards warmer and arid places. Although they also respond to winter precipitation and precipitation seasonality, in this climate they can occur in a large range of these variables so its value as indicator is lower.

For the climate with more winter rain we found two ecological indicators based on functional groups. Under these climatic

conditions, both cyanolichens and broad lobed lichen species increase in relative abundance can be used to track increases in isothermality. The opposite trend in their relative abundance can be used to track increases in temperature seasonality. Finally, foliose broad lobed can also be used in this climate to track precipitation summer decreases, as this functional group will decrease its relative abundance under increasing summer precipitation levels.

In reference to the whole gradient studied, because several limiting factors act simultaneously, only two functional groups responded. Cyanolichens lichens relative abundance in the whole gradient will be limited to places with low summer precipitation and low temperature annual range, thus changes in its relative abundance can be used as an ecological indicator to track changes in both these climate variables. On the other hand, the relative abundance of the resistant cyanolichens can be used to track changes in winter precipitation, aridity and summer temperatures. In this large gradient this functional group can only found in places with high summer temperature, aridity and low winter rain, and its relative abundance will be increasingly higher as we move towards more arid regions.

CONCLUSIONS

For the first time we were able to find a set of specific ecological indicators of changes in climate along a spatial gradient based on lichen functional groups. The developed indicators have the potential to be applied globally, but contrarily to what we expected, their response was not unique at a global scale, but specific to the climatic limiting factor. Because lichen functional group-based indicators respond to the most limiting climate factors, and these vary as we move on the globe, its application must be stratified by homogenous areas sharing similar climate limiting factors. As previously found (chapter four), lichen functional groups responded consistently to temperature across climates. However, response to water was limited to climates where water was a limiting factor, suggesting that lichens follow partly the global vegetation limitations in response to the complex interactions between temperature, water and radiation (Churkina & Running, 1998). These findings enhance the potential of lichen functional diversity as ecological indicators of spatial changes in climate, as they seem to integrate and illustrate the complex and diverse control imposed by climate on vegetation activity, thus warranting a more comprehensive understanding of the effects of climate change (Nemani *et al.* 2003).

These results are very crucial under a climate change perspective. If the ecological indicators found here are validated over time,

than a space-for-time proxy can be used to predict the effects of forecast changes due to climate change. Future work should focus on validating if lichen functional groups responding to each climate over space, are also those responding to shifts in climate over time, so they can be used as ecological indicators of climate change at a global scale. This would be of the utmost importance. Lichen functional groups specific response to seasonal changes in temperature and its range, as well as to seasonal changes in the patterns of precipitation and the levels of relative humidity may be a valuable tool for the future. The seasonal patterns of temperature, precipitation and relative humidity, though the latter with less confidence, are expected to change (Collins *et al.* 2013), thus these indicators may be crucial to help us signal subtle changes already happening in the present.

The global application of this method is limited to places where trees exist. Future work should also contemplate the development of a similar method to be applied on soil crusts in regions where tree cover is very low or inexistent. In these areas dominated by biological soil crusts, lichen functional diversity has also a high potential to be used as ecological indicator of climate.

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chapter six

Ecological indicators: validating space with time to track the effects of climate change

publication

Matos P., Pinho P., Nunes A., Soares C., Soares A.M.V.M., Branquinho C. In preparation.

abstract

Due to climate change, severe social and ecological impacts have been observed and are expected to worsen. For this reason efforts have been gathered to understand and quantify how ecosystems are affected by and respond to these changes. Hence, we need universal biodiversity metrics and ecological indicators capable of evaluating the effects of global change on ecosystems structure and function. Recently, lichen functional diversity was shown to respond to climate gradients. Nonetheless, since this work was based on a space-for-time proxy, if we want to use them as ecological indicators to signal changes over time we need to validate them through time as well.

Here we analyse epiphytic lichen communities over the course of fifteen years with the objective of validating the ecological indicators of climate change previously identified in space, so they can to be used over time. Several biodiversity-based metrics were tested to know which perform better over time, namely functional diversity, species richness, abundance and community structure.

Our results show that space is a good substitute for time, and validate lichen functional diversity as a sensitive ecological indicator of climate change. Lichen community composition changed significantly over the course of fifteen years (1994-2010), and these shifts were strongly related to subtle shifts in climate. The compositional changes observed were mediated by changes in lichen functional composition, but not by species richness. This highlights that species richness is not enough, and metrics including abundance, community structure and functional diversity are needed to track the effects of climate change. Lichens responded to subtle changes in climate not yet depicted by the currently used climate change metrics. This suggests that they might have the potential to be used as early warning indicators of classic climate metrics related to temperature and precipitation. Hence, future work should be devoted to explore this potential and to understand if they are able to signal these changes before other less responsive components of the ecosystems.

keywords

Space-for-time, climate change, functional groups, functional diversity, lichens

introduction

Severe social and ecological impacts have been observed and are expected to worsen due to climate change (IPCC 2014). For this reason, efforts have been gathered to track and predict climate change variables and drivers. This is essential for attribution of causes, for better predictions and to define risk assessments, vulnerabilities and adaptation measures (GCOS 2004). However, tracking and monitoring climate change effects

necessary implies more than just tracking and monitoring climate driver's change over time. It is imperative to understand and quantify how ecosystems' functioning is affected by and respond to these changes (a 2ºC change won't have the same effect in alpine and desert ecosystems). Climate change acts on a global scale and much of the political measures adopted in its response are usually also determined in the international sphere (UN conventions on Biodiversity, Climate Change or Desertification and Land Degradation are an example). Hence, we need universal biodiversity metrics and ecological indicators capable of evaluating the effects of global change on ecosystems structure and function (Pereira *et al.* 2013), to preferentially early warn us before it is too late to take action. These indicators are essential to track global trends of climate change effects, to assess the effectiveness of the political measures adopted in its response and to prepare and define new strategies for the future (Branquinho, Matos & Pinho 2015).

There is a growing consensus about the scale and metrics to be used when tracking climate and other global change drivers' effects. A recent work has brought some light into the importance of considering the different spatial scales of biodiversity to better understand trends in response to global change (McGill *et al.* 2015). This is important because biodiversity trends may be different when different scales are considered. The choice of metrics to use is also fundamental. Species richness metrics are essential to tell the compelling story about biodiversity loss, but their response to climate change is not as fast as other metrics' response (Balmford, Green & Jenkins 2003; Dornelas *et al.* 2014). Metrics incorporating measures of species abundance and community structure are more responsive (Balmford, Green & Jenkins 2003; Pereira, Navarro & Martins 2012; Pereira *et al.* 2013; Dornelas *et al.* 2014; McGill *et al.* 2015). Functional diversity metrics should also be contemplated. They are better than species related metrics to quantify response to global change, and can provide a stronger link with ecosystems functioning and their services provision (Díaz & Cabido 1997; Díaz *et al.* 2007; Suding *et al.* 2008; Lavorel *et al.* 2011; Mouillot *et al.* 2012). Thus, ecological indicators of climate should be able to perform from local to global spatial scales, and the metrics used to depict their trends in response to climate change should contemplate not only species richness, but also incorporate measures of abundance, community structure and functional diversity.

Epiphytic lichen diversity has been successfully used all over the globe to track the effects of global change related with several atmospheric pollutants (Augusto, Máguas & Branquinho 2013; Barros *et al.* 2015), land use change (Pinho *et al.* 2008), nitrogen pollution (Geiser *et al.* 2010; Pinho *et al.* 2011) or biodiversity

assessment and conservation (Nascimbene, Marini & Nimis 2007; McMurray, Roberts & Geiser 2015). More recently, researchers started also to explore their potential to indicate changes in climate (Geiser & Neitlich 2007; Marini, Nascimbene & Nimis 2011; Matos *et al.* 2015). A work in the Netherlands was most probably the instigator of this research line (van Herk, Aptroot & Van Dobben 2002; Aptroot & Van Herk 2007). This work reported lichen compositional changes over a short-time period (5 years), attributable to global warming. These research efforts, although still sparse, have recently culminated into the development of ecological indicators of climate change based on lichen functional diversity (chapter five). Nonetheless, this work was based on space-for-time proxy, and its validation over time is still lacking.

Here we analyse lichen epiphytic community change over time with the objective of validating over time ecological indicators of climate change previously developed based on a space-for-time substitution (chapter five). At four time points in the course of fifteen years we assessed lichen species composition in 22 sampling sites in southwestern Portugal. Several reasons underpin the choice of this area: i) local population awareness of an increased number of days with dew condensation in the morning; ii) this climate fluctuation was not yet detected by traditional climate metrics; iii) the area is classified as semi-arid and as susceptible to desertification and land degradation (Costa, Santos & Pinto 2012); iv) a long-term study with lichen diversity was available. Changes over time were investigated using functional diversity metrics that were the basis of previously developed lichen-based ecological indicators, and also using metrics of species richness, abundance and community structure to obtain an integrated picture of these indicators response to climate shifts over time. Temperature, precipitation and relative humidity from a local meteorological station were used to compute bioclimatic variables to relate to the observed temporal changes in lichen communities with ongoing climate fluctuations.

materials & methods

STUDY SITE

This study was conducted in southwestern Europe (Portugal, Alentejo region) in a holm-oak open woodland on an area of around 228 km^2 (Fig. 1). This region has a typical Mediterranean seasonal climate characterized by dry hot summers and cold rainy winters. Mean annual temperature is 16.2 ºC and annual mean precipitation 559 mm (average of all the sites calculated with data from WorldClim relative to 1960 - 1990). This area is characterized as semi-arid, and has been classified as susceptible to desertification and land degradation (Rosário 2004).

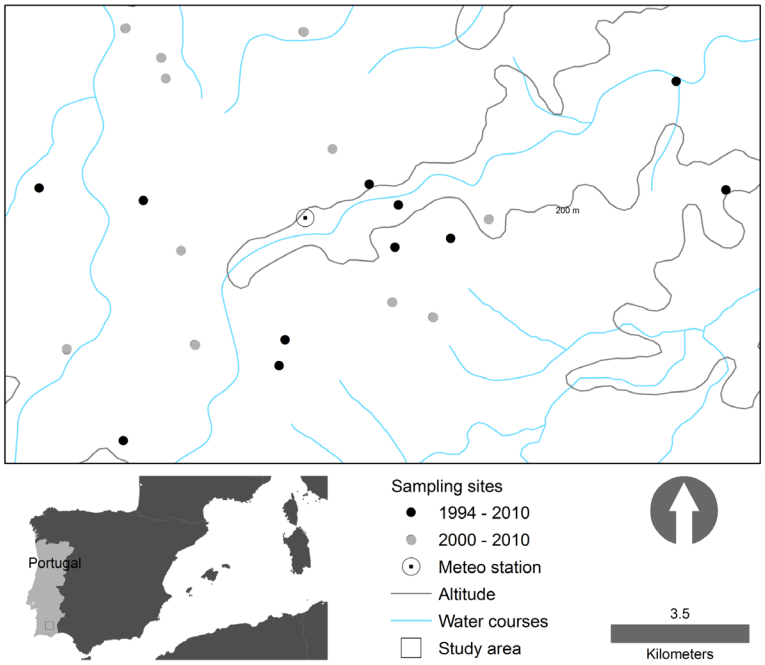

fig. 1. Map of the study area showing sampling sites distribution and the meteorological station locations.

Eleven sampling sites were established in 1994 to track changes in epiphytic lichen communities over time at a more or less regular 5-year interval. In 2000, the number of sites was enlarged to 22, and those sites have been followed ever since (2000, 2005 and 2010).

LICHEN SAMPLING

Over these fifteen years, two different methodologies were used to assess lichen epiphytic diversity. In 1994 and 2000 only species presence was registered. At this time, for each site, holm-oak (*Quercus ilex*) tree trunks were surveyed for lichens presence at a height between 50 cm and 2 m.

Since 2005, in addition to the same visual inspection, lichen frequency was also recorded following the European method (Asta *et al.* 2002). A minimum of 1 and a maximum of 5 trees were sampled in each site, depending on the number of available trees complying with protocol requirements. A 10 cm x 50 cm grid divided in five 10 cm squares was placed on the four main cardinal directions of the trunks and all species occurring inside the quadrats were registered. The uppermost part of the sampling grid was placed at 1.5 m from the ground and height was adjusted to a maximum of 2 m whenever trunk characteristics were unsuitable for sampling. A total of 140 species were identified over these fifteen years. Species impossible to identify in the field were collected and taken to the laboratory for identification.

These measurements over time resulted in two different datasets:

table 1. Climate variables calculated from raw data collected in a local meteorological station.

Main variable	Type		Worldclim code	Variable
Temperature (ºC)	Annual		Bio 1	Annual Mean Temperature (AMT)
	Range		Bio 2	Mean Diurnal Range (MDR)
			Bio 7	Temperature Annual Range (TAR)
			Bio 3	Isothermality (I)
	Seasonal	Seasonality	Bio 4	Temperature Seasonality (TS)
		Summer	Bio 9	Mean Temperature of Driest Quarter (MTDQ)
			Bio 10	Mean Temperature of Warmest Quarter (MTWMQ)
			Bio 5	Max Temperature of Warmest Month (MAXT)
		Winter	Bio 8	Mean Temperature of Wettest Quarter (MTWTQ)
			Bio 11	Mean Temperature of Coldest Quarter (MTCQ)
			Bio 6	Min Temperature of Coldest Month (MINT)
Precipitation (P)	Annual		Bio 12	Annual Precipitation (AP)
	Seasonal	Seasonality	Bio 15	Precipitation Seasonality (PS)
		Summer	Bio 17	Precipitation of Driest Quarter (PDQ)
			Bio 18	Precipitation of Warmest Quarter (PWMQ)
			Bio 14	Precipitation of Driest Month (PDM)
		Winter	Bio 16	Precipitation of Wettest Quarter (PWTQ)
			Bio 19	Precipitation of Coldest Quarter (PCQ)
			Bio 13	Precipitation of Wettest Month (PWT)
Relative humidity	Annual			(RH)
	Number of days			Number of days with RH higher than 95% (RH95)

i) one considering lichens species presence in four time points - 1994, 2000, 2005 and 2010; ii) another accounting with species frequency in two time points - 2005 and 2010.

CLIMATE DATA

Climate data was retrieved from a local meteorological station (Fig. 1, IPMA station 264). This meteorological station is operational only since 2000, so there is no data available prior to that year. Minimum and maximum weekly temperatures and weekly precipitation were used to calculate a set of 19 bioclimatic variables representing range, seasonal and annual variation of temperature and precipitation, recreating those used in WorldClim (see www.worldclim.org for details on variables with codes Bio1 to Bio 19, Table 1). Annual mean relative humidity in this time interval was also calculated. Because these metrics revealed unresponsive over these fifteen years and local populations were aware of a general increase in the number of days with dew condensation in the morning, the number of days with relative humidity higher than 95 % was also calculated. These variables

table 2. Traits and related functional groups (Nimis & Martellos, 2008; Smith *et al.* 2009).

Trait	Functional group	Description	Symbol
Type of primary photobiont	Chloroccocoid	With Chloroccocoid (Green algae)	Ch
	Trentepohlioid	With *Trentepohlia* (Green algae)	Tr
	Cyanolichens	With Cyanobacteria	Cy
	Resistant cyanolichens	A specific set of lichens with cyanobacteria and jelly structure (homoiomerous), that are mostly found in dry climate	Cyj
Growth form	Crustose	Firmly and entirely attached to the substrate by the lower surface	Cr
	Leprose	Like crustose but surface thallus with a granular mass appearance and always decorticated	Lp
	Squamulose	Composed of small scales	Sq
	Foliose narrow-lobed	Partly attached to the substrate with a leaf-like form and narrow lobes	Fn
	Foliose broad-lobed	Same as Foliose narrow-lobed but with broad lobes	Fb
	Fruticose	3D-like structure, attached by one point to the substrate with the rest of the thallus standing out from the surface of the substrate	Fr
Size	Microlichens	With crustose or leprose growth form.	Mi
	Macrolichens	With growth form other then crustose and leprose.	Ma

were computed on a yearly basis until 2011.

LICHEN FUNCTIONAL DIVERSITY

This work was based on three lichen response traits and its respective functional groups: main type of photobiont, growth form and size (see Table 2 for description of its functional groups). These traits were previously identified as indicators of climate change along spatial gradients, due to their responsiveness to climate (Matos *et al.* 2015). Trait classification was based on the Italian database (Nimis & Martellos 2008) and lichen floras, if absent from the database (Llop 2007; Smith *et al.* 2009; Giralt 2010; Carvalho 2012).

Community weighted means (CWM) for each functional group were calculated for both presence and abundance datasets. Because all traits are categorical the CWM represents the mean proportion of each functional group (FG) in the community either weighted by the number of species (in the case of presence data), or by the abundance of species belonging to that functional group (Lavorel *et al.* 2008) (CWM of each FG × site). Presence data in each site was combined with species trait data to calculate the community level weighted mean (CWM-P) for the four time points (2000, 2005 and 2010). For two time points, 2005 and 2010, the average species frequencies in each site (sites x species frequency) was combined with species trait data to calculate the CWM based on the abundance (CWM-A).

In addition, for each time point the number of species belonging

to each functional group were also computed.

STATISTICAL ANALYSIS

The first analysis focused on assessing changes in lichen species composition over time. These differences were described using non-metric multidimensional scaling (NMS) based on Bray-Curtis distance (PC-ORD, version 6.08). This distance measure is one of the most effective measures of species dissimilarities and the recommended distance measure for community data analysis (McCune, Grace & Urban 2002). The best NMS solution was chosen from 500 runs, each starting randomly (500 iterations per run), and evaluated with a Monte Carlo test (250 runs with randomized data, considered significant for $p < 0.05$). The coefficients of determination (r^2) between the original plot distances and distances in the final ordination solution were calculated to assess how much of the lichen community variability was represented by the NMS axes (McCune, Grace & Urban 2002). Compositional changes were assessed with presence data for four time points (1994, 2000, 2005 and 2010, eleven sites) and with abundance data for two data points (2005 and 2010, 22 sites). From 2000 to 2010 datasets have 22 sites. Compositional changes over this time and considering the 22 sites were also analysed, and resulted similar to those with eleven sites for the four time periods, thus they are not shown. Climate and functional variables were overlaid on the NMS ordination as correlation vectors. Because climate data is only available for the period of 2000 to 2010, compositional changes for this period are also shown. Individual correlations between these variables and NMS site scores were determined using Spearman correlations (correlations were considered significant for $p < 0.05$, Statistica version 13). Permutational analysis of variance (PERMANOVA) was used to test compositional changes over time (PC-ORD version 6.08). This analysis is very similar to ANOVA, but allows to test differences in species composition rather than species numbers (Anderson 2001). Successional vectors were used to illustrate the direction of compositional change over years (McCune, Grace & Urban 2002).

To assess changes in species richness and diversity, the number of species and Shannon's diversity index were calculated for each sampling site in each time point (PC-ORD version 6.08). Shannon's diversity index was calculated both with presence and abundance datasets. Because results were identical, only those for presence data are shown.

To determine if each biodiversity metric (species richness, Shannon's diversity index, CWM-P, CWM-A and number of species of each FG) and each climate metric changed significantly

over time, we used general linear models (GLM), with time as a continuous explanatory variable and each metric as response variables (Statistica software, version 13). Changes in climate over time were based on 12 time points (2000 to 2011). Changes in biodiversity metrics over time were determined for the period of 1994 to 2010 using the set of 11 sites, and for the period of 2000 to 2010 for the set of 22 sites. Because trends are similar, only those relative to 1994 to 2010 are presented. GLMs were also used to determine if changes in biodiversity metrics were related to changes in climate. In this case, climate variables with significant change over time were used as continuous explanatory variables (with data relating to the four data points of lichen measurements) and biodiversity metrics as response variables. As climate data is only available for the period of 2000 to 2010, analyses were only computed for this time interval with the set of 22 sampling sites. For all models, only those with $r^2 \geq 0.20$ were discussed, as those with lower fits might have a low ecological meaning.

results

CLIMATE SHIFTS OVER TIME

There were no significant trends in the traditional climate metrics over the period of 2000 to 2011 (19 bioclim variables and mean RH, Table 3). Since local populations perceived a change in climate, we searched for other climatic variables that could depict these shifts. We found that the number of days with relative humidity higher than 95 % has been increasing significantly from 2000 to 2011 (Table 3), an increase particularly noticeable since 2005 (Fig. 2).

SPECIES RICHNESS AND DIVERSITY

There were no significant changes in species richness or Shannon's diversity index over time based on species presence in 11 sites from 1994 to 2010 ($r^2 < 0.01$, $p = 0.982$, Fig. 3).

COMMUNITY COMPOSITION

Lichen species composition in terms of species presence changed significantly in the eleven sites sampled from 1994 to 2010 ($F_{3, 43} = 94.19$, $P < 0.001$, $N = 44$, Fig. 4 A). Overall species composition in 1994, 2000 and 2005 was more similar among them, than with species composition in 2010. Although compositional changes were observed between 1994 and 2000, and between 2000 and 2005, the transition that occurred between 2005 and 2010 appears to be the largest (Fig. 4 B). In this period of time, compositional changes were also evident

table 3. Change in climate metrics related to temperature and precipitation (annual mean, range and seasonality) over time. Results were obtained with GLMs using yearly averages from 2000 to 2011. Signal represents sign of the relationship. n.s. indicates non-significant relationships. Climate variables can be decoded in Table 1.

Variables	Signal	r^2	F	p
HR95	+	0.79	38	< 0.001
AMT		0.07	n.s.	n.s.
MDR		0.02	n.s.	n.s.
I		0	n.s.	n.s.
TS		0	n.s.	n.s.
MAXT		0.05	n.s.	n.s.
MINT		0	n.s.	n.s.
TAR		0.02	n.s.	n.s.
MTWTQ		0.09	n.s.	n.s.
MTDQ		0.03	n.s.	n.s.
MTWMQ		0.02	n.s.	n.s.
AP		0.11	n.s.	n.s.
PWTM		0.03	n.s.	n.s.
PDM		0.04	n.s.	n.s.
PWMQ		0.15	n.s.	n.s.
PCQ		0.1	n.s.	n.s.
AMHR		0.21	n.s.	n.s.

when considering species abundance (Fig. 4 C and D).

CHANGES IN FUNCTIONAL COMPOSITION

Lichen functional trait composition showed significant trends of change over time related to changes in some functional groups of main type of photobiont, growth form and size (Table 4, Fig. 2). The compositional changes over this period were reflected both in terms of the CWM-P from 1994 to 2010, and on the CWM-A from 2005 to 2010. The number of species of some functional groups showed significant trends over time, but the low r^2 of the models is indicative of low ecological relevance, so they will not be treated as significant trends. These compositional changes over time were related to changes in lichens main type of photobiont. Since 1994, the CWM-P of trentepohlioid species increased. This increase seemed to be particularly marked since 2005, as also evident in the CWM-A of trentpohlioid species in the period of 2005 to 2010. Conversely, this period corresponded to a decline in the CWM-A of chlorococcoid lichens. Lichen compositional changes reflected also significant changes in growth form functional groups. Since 1994, the CWM-P of crustose increased, while the opposite trend was observed for foliose broad lobed species. These trends were also observed from 2005 to 2010 in the CWM-A, and for this period the CWM-A of fruticose species also decreased significantly. Lichens classification according to size was also associated with compositional changes. Since 2000, CWM-P of macrolichens decreased and the CWM-P of

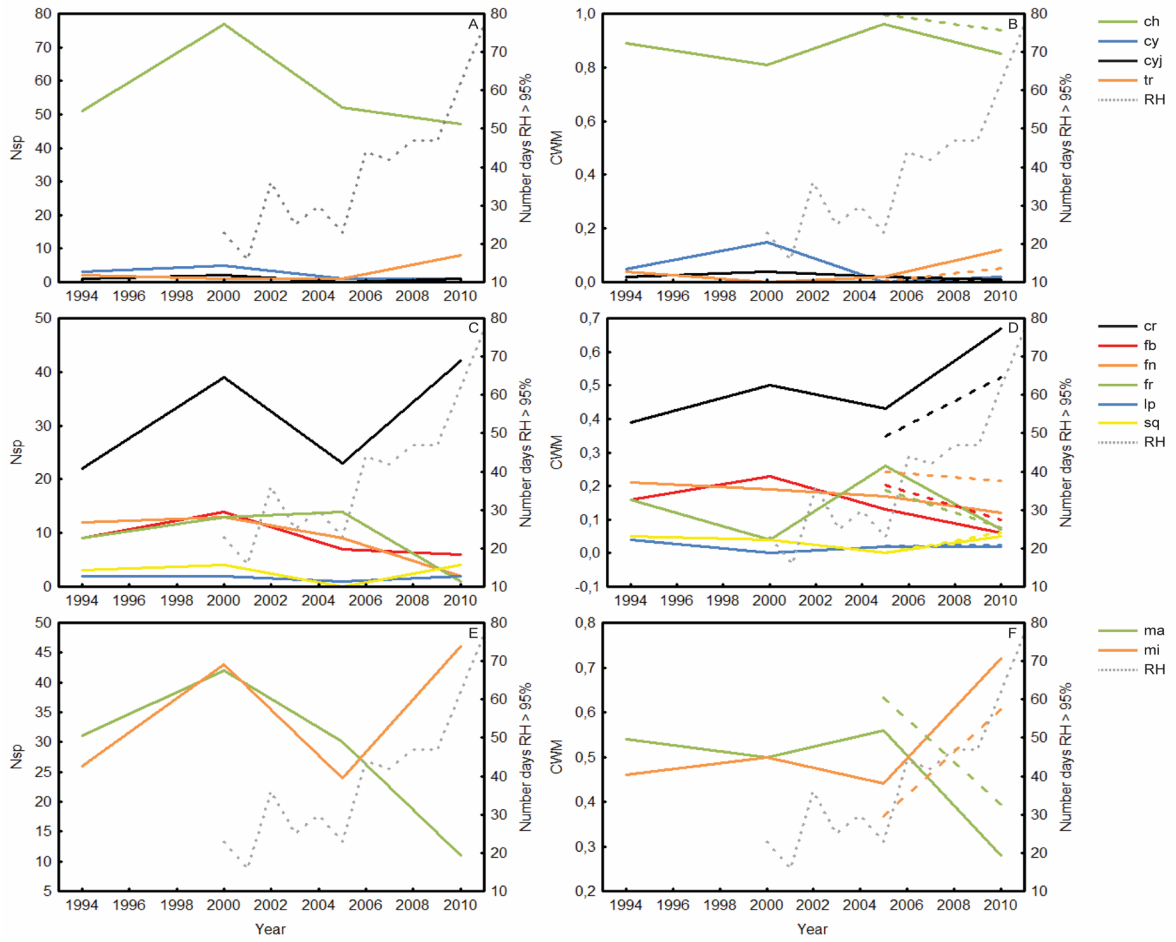

fig. 2. Changes in the average number of lichens (Nsp) and on average community weighted mean (CWM) of each functional group of main type of photobiont (A, B), growth form (C, D) and size (E, F) over time (1994, 200, 2005 and 2010). Changes over time (yearly from 2000 to 2011) in the number of days with relative humidity higher than 95% are also shown (grey dotted line). CWM based on presence is represented by solid lines, and CWM based on abundance with dashed lines.

microlichens increased. The CWM-A from 2005 to 2010 showed also the same trend.

LICHEN SHIFTS AND ITS RELATION WITH CLIMATE FLUCTUATION

Lichen species composition based on species presence from 2000 to 2010 and based on abundance in the period of 2005 to 2010 seemed to follow the increasing trend in the number of days with relative humidity higher than 95 % (Fig. 4 E and F).

Lichen functional trait composition showed also significant changes with the number of days with relative humidity higher than 95 % (RH95) related to changes in some functional groups of main type of photobiont, growth form and size (Table 4). The number of trentepohlioid and crustose species showed an increase with RH95, while the reverse trend was observed for fruticose lichens. In reference to the CWM-P and CWM-A,

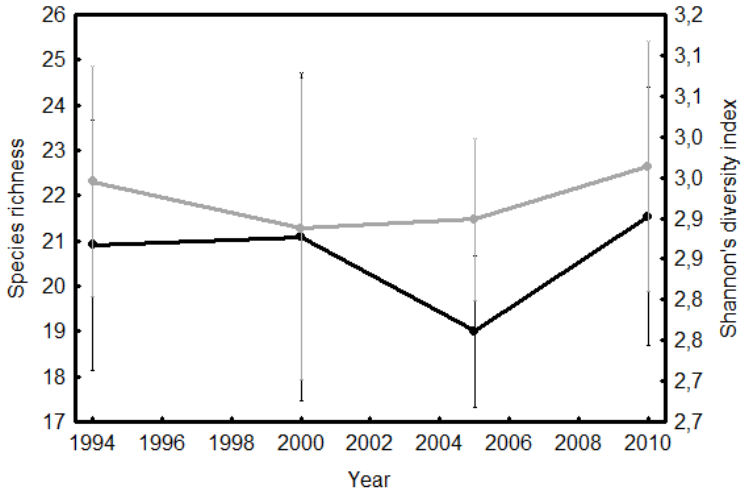

fig. 3. Changes in species richness (black circles and solid line) and Shannon's diversity index (grey circles and solid line) over time for in eleven sampling sites. Whiskers represent ± SE.

changes followed overall the same trend pattern as changes over time, but with stronger relationships in the case of CWM-P.

discussion

Recent studies developed indicators of climate based on lichen functional diversity using a space-for-time proxy (chapter five). Subtle shifts on climate perceived by local populations presented the perfect opportunity to test these indicators over time. Because no significant changes were detected on traditional climate metrics of temperature, precipitation (annual mean, range and seasonality) and relative humidity measured in the study area from 2000 to 2011, we searched for other climate metrics that could depict the changes perceived by local populations. After testing several other metrics, we observed a significant increase in the number of days with relative humidity higher than 95 % during this period. Some lichen functional groups were previously identified as indicators of change in mean trends of relative humidity in climatic gradients in space, and those were the ones we expected to shift over time if space substitution works. As expected, lichen functional traits that responded to climate in space, shifted in response to the increase in the number of days with relative humidity higher than 95 % measured over time. These shifts were observed even in the absence of detectable changes in its annual mean trend, confirming our initial hypothesis that space for time substitution is possible in this case. Our work validates these lichen functional diversity indicators over time, cementing its establishment as ecological indicators to track climate change.

Results showed significant shifts in functional composition over time related to the main type of photobiont, growth form and size, and these changes followed the marked increasing trend in the number of days with relative humidity higher than 95 %. These

table 4. Changes in number of species and in community weighted mean values (CWM) over time and with the number of days with relative humidity higher than 95% (RH95). Relationships are shown for the sets of 11 (1994 to 2010) and 22 sampling sites (2000 to 2010) in the case of number of species and CWM based on presence (CWM-P). CWM based on abundance (CWM-A) refers only to two time points (2005 and 2010). Change of climate over time is also shown. Results were obtained with GLMs. Signal represents sign of the relationship. n.s. indicates non-significant relationships. Models with r^2 higher than 0.20 are highlighted in bold. See Table 2 to decode lichen functional groups.

		Based on 11 sites							
		Time				RH95			
Variables	Functional group	Signal	r^2	F	p	Signal	r^2	F	p
No. species of each functional group	Ch		0.01	n.s.	n.s.		0.01	n.s.	n.s.
	Cy	-	0.14	7	0.012		0.04	n.s.	n.s.
	Cyj		0.02	n.s.	n.s.		0	n.s.	n.s.
	Tr		0.07	n.s.	n.s.	**+**	**0.22**	**11.9**	**0.001**
	Cr		0.08	n.s.	n.s.	+	0.19	9.6	0.003
	Fb		0.06	n.s.	n.s.		0.07	n.s.	n.s.
	Fn		0.02	n.s.	n.s.		0.01	n.s.	n.s.
	Fr		0.05	n.s.	n.s.		0.14	n.s.	n.s.
	Lp		0	n.s.	n.s.		0	n.s.	n.s.
	Sq	-	0.1	4.4	0.041		0	n.s.	n.s.
	Ma		0.05	n.s.	n.s.		0.09	n.s.	n.s.
	Mi		0.04	n.s.	n.s.	+	0.14	7.1	0.011
CWM-P	Ch		0.04	n.s.	n.s.	**-**	**0.23**	**12.7**	**< 0.001**
	Cy	-	0.15	7.4	0.009		0.05	n.s.	n.s.
	Cyj		0.03	n.s.	n.s.		0	n.s.	n.s.
	Tr	+	0.14	7.1	0.011	**+**	**0.35**	**22.8**	**< 0.001**
	Cr	**+**	**0.21**	**11**	**0.002**	**+**	**0.36**	**23.9**	**< 0.001**
	Fb		0.12	5.6	0.023	**-**	**0.22**	**11.5**	**0.001**
	Fn		0.02	n.s.	n.s.		0.03	n.s.	n.s.
	Fr		0.04	n.s.	n.s.	-	0.16	7.8	0.008
	Lp		0.01	n.s.	n.s.		0	n.s.	n.s.
	Sq		0.04	n.s.	n.s.		0	n.s.	n.s.
	Ma	-	0.11	5.3	0.026	**-**	**0.29**	**17.2**	**< 0.001**
	Mi	+	0.11	5.3	0.026	**+**	**0.29**	**17.2**	**< 0.001**

traits were already identified as responsive to mean annual relative humidity (chapters four and five), namely trentepohlioid lichens, foliose narrow, fruticose, crustose, macro and microlichens. Our results show that these functional groups are sensitive to subtle changes in relative humidity over time and that they can be used as ecological indicators of climate change. As expected from previous works, these lichen functional compositional changes observed over time did not coincide with significant changes in species richness or Shannon's diversity index. Despite some local declines in species richness and diversity, this trend is still not significant (Dornelas *et al.* 2014), probably due to a time lag in their response to climate change (Balmford, Green & Jenkins 2003). Despite back and forth transitions in lichen composition observed from 1994 to 2005, we found evidence of large directional species compositional change since 2005. These

table 4. (continuation).

		Based on 22 sites							
		Time				RH95			
Variables	Functional group	Signal	r^2	F	p	Signal	r^2	F	p
No. species of each functional group	Ch		0.04	n.s.	n.s.		0.01	n.s.	n.s.
	Cy		0.08	n.s.	n.s.		0.02	n.s.	n.s.
	Cyj		0.01	n.s.	n.s.		0.06	n.s.	n.s.
	Tr	+	0.15	7.3	0.009	**+**	**0.30**	**26.9**	**< 0.001**
	Cr	+	0.1	7.3	0.009	**+**	**0.22**	**18.5**	**< 0.001**
	Fb	-	0.16	12.3	0.001	-	0.14	10	0.002
	Fn		0.01	n.s.	n.s.		0.01	n.s.	n.s.
	Fr	-	0.19	14.6	< 0.001	**-**	**0.21**	**17.1**	**< 0.001**
	Lp			n.s.	n.s.		0	n.s.	n.s.
	Sq	-	0.06	4.3	0.042		0	n.s.	n.s.
	Ma	-	0.16	12.1	0.001	-	0.16	12.1	0.001
	Mi		0.06	n.s.	n.s.	+	0.19	15	< 0.001
CWM-P	Ch	-	0.14	10.0	0.002	**-**	**0.31**	**29.4**	**< 0.001**
	Cy	-	0.07	4.9	0.03		0.01	n.s.	n.s.
	Cyj		0.02	n.s.	n.s.		0.09	6.2	0.015
	Tr	**+**	**0.22**	**17.7**	**< 0.001**	**+**	**0.38**	**39.0**	**< 0.001**
	Cr	**+**	**0.36**	**35.7**	**< 0.001**	**+**	**0.49**	**62.1**	**< 0.001**
	Fb	**-**	**0.22**	**17.8**	**< 0.001**	**-**	**0.33**	**31.7**	**< 0.001**
	Fn			n.s.	< 0.001		0.01	n.s.	n.s.
	Fr	-	0.13	9.7	0.003	**-**	**0.25**	**21.6**	**< 0.001**
	Lp			n.s.	n.s.		0	n.s.	n.s.
	Sq		0.01	n.s.	n.s.		0.03	n.s.	n.s.
	Ma	**-**	**0.24**	**19.8**	**< 0.001**	**-**	**0.45**	**52.7**	**< 0.001**
	Mi	**+**	**0.24**	**19.8**	**< 0.001**	**+**	**0.45**	**52.7**	**< 0.001**
CWM-A	Ch	**-**	**0.31**	**19.2**	**< 0.001**	**-**	**0.31**	**19.2**	**< 0.001**
	Cy		0.02	n.s.	n.s.		0.02	n.s.	n.s.
	Cyj	+	0.15	7.7	0.008	+	0.15	7.7	0.008
	Tr	**+**	**0.28**	**16.2**	**< 0.001**	**+**	**0.28**	**16.2**	**< 0.001**
	Cr	**+**	**0.24**	**13.6**	**0.001**	**+**	**0.24**	**13.6**	**0.001**
	Fb	**-**	**0.29**	**17.2**	**< 0.001**	**-**	**0.29**	**17.2**	**< 0.001**
	Fn		0.01	n.s.	n.s.		0.01	n.s.	n.s.
	Fr	**-**	**0.21**	**10.9**	**0.002**	**-**	**0.21**	**10.9**	**0.002**
	Lp			n.s.	n.s.		**0**	**n.s.**	**n.s.**
	Sq	**+**	**0.29**	**17.0**	**< 0.001**	**+**	**0.29**	**17.0**	**< 0.001**
	Ma	**-**	**0.44**	**33.2**	**< 0.001**	**-**	**0.44**	**33.2**	**< 0.001**
	Mi	**+**	**0.44**	**33.2**	**< 0.001**	**+**	**0.44**	**33.2**	**< 0.001**

findings support the increasing amount of works highlighting species compositional shifts as a more responsive metric than species richness to track the effects of global change (Balmford, Green & Jenkins 2003; Pereira *et al.* 2010; Pereira *et al.* 2013; Dornelas *et al.* 2014; McGill *et al.* 2015). The large unidirectional change in lichen species composition observed since 2005

followed the significant increase in the number of days with relative humidity higher than 95 %, that almost tripled since then. Lichens are poikilohydric organisms that reflect temporally and spatially the various sources of hydration (Gauslaa 2014), and their response to spatial changes in relative humidity had already been demonstrated (chapters four and five). Our work suggests that they also respond temporally to relative humidity changes in a short period of time (less than 5-year interval), and that they are sensitive to fine scale changes of relative humidity, undetected by commonly used climate metrics (i.e. annual mean). These results reinforce the need to contemplate abundance, community structure and functional biodiversity metrics, as species richness metrics are not enough to evaluate the effects of climate change.

The type of primary photobiont and growth form are frequently identified as key traits responsible for mediating lichen relationship with water sources, placing lichens among the most responsive organisms to moisture (Gauslaa 2014). Our results show that under increasing levels of relative humidity, lichens with trentepohlioid photobionts increase and chlorococcoid decrease. On the other hand, we observed an increasing dominance of crustose species, while fruticose and foliose broad decreased. These growth form shifts under increasing levels of relative humidity translated into a general trend showing that size matters: microclichens increased and macrolichens decreased. These shifts in main type of photobiont, growth forms, and ultimately size, are in some way parallel to what happens in climate gradients in humid warm tropics. In lowland humid tropical forests small size crustose lichens dominate, and foliose and fruticose lichens appear only more frequently in mountain and pre-mountain rain forests (Lakatos, Rascher & Büdel 2006). These patterns are particularly marked in these forests understory, and may be related to highly saturating water conditions, low light intensity and negative carbon balances as a result of high nocturnal temperatures, with whom crustose species seem to better cope with (Cowan, Lange & Green 1992; Zotz, Schultz & Rottenberger 2003; Lakatos, Rascher & Büdel 2006; Pardow, Hartard & Lakatos 2010). At the same time, the warm humid conditions in the tropics seem to favor the dominance of trentepohlioid species (Rundel 1978; Rindi & Lopez-Bautista 2008). With the exception of light conditions, which were constant and non-limiting in our work, the other factors may partly explain the trends observed in our study.

This work has several implications under an early warning perspective. While no detectable patterns of change were seen in classical metrics of climate, after searching for other less traditional climate variables we found a significant increase in the number of days with relative humidity higher than 95 % in

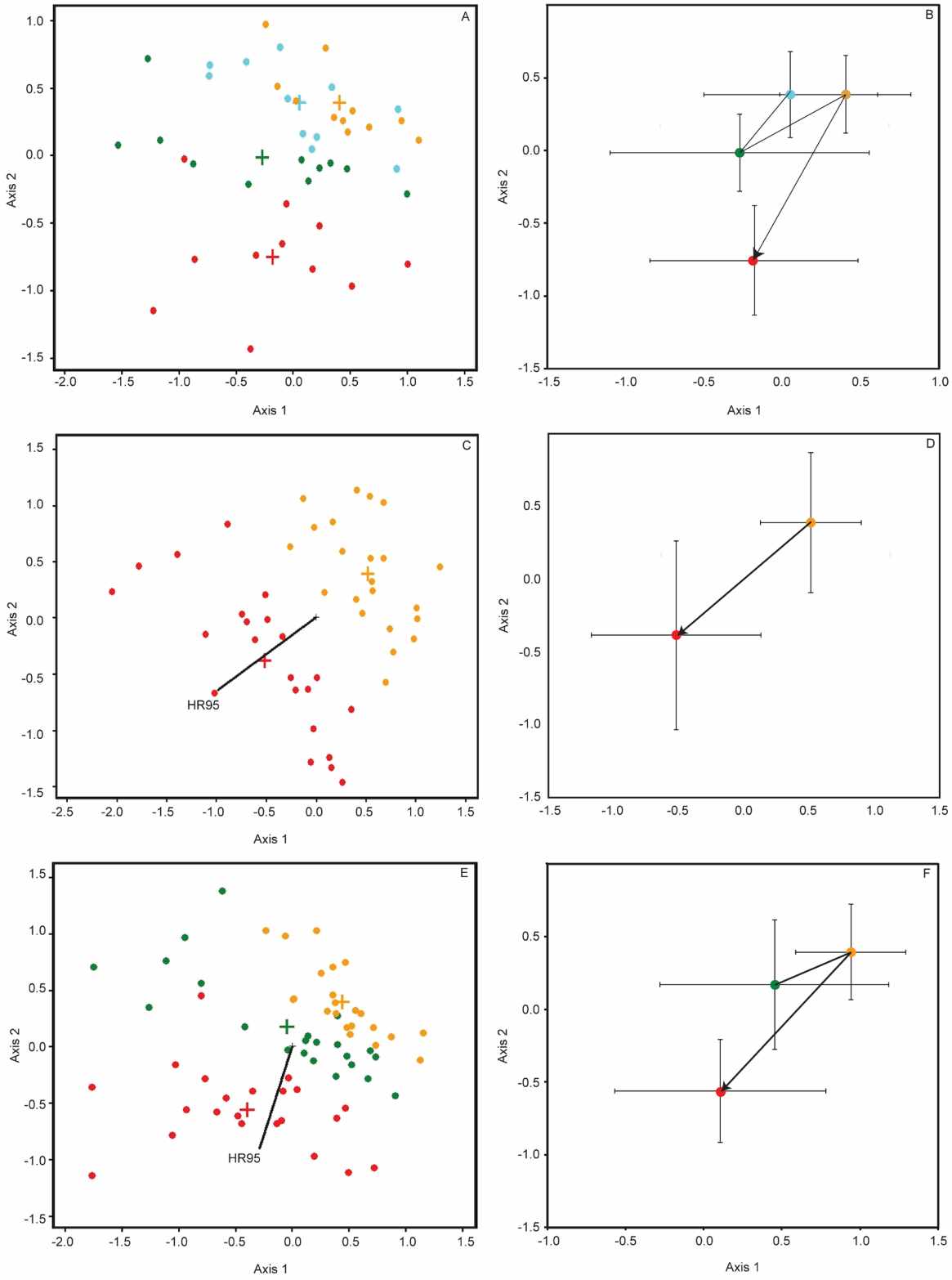

fig. 4. Differences in lichen community composition among sampling sites based on presence of species (A, B, E, F) and abundance (C and D). A and B refer to changes from 1994 to 2010, and E and F to changes from 2000 to 2010. Differences based on abundance refer to changes from 2005 to 2010 (C, D). Symbols in graphs B, D and F indicate the mean NMS scores (±SD) of the overall plots from A, C and E, respectively, in a given year, and successional vectors indicate the mean direction of change. Symbols closer to each other are more similar to each other than those further apart. Vectors on figures C and E represent significant relation with the number of days with relative humidity higher than 95% (RH95). Sites (circles) and year centroids (+) are color-coded according to time point: 1994 - blue; 2000 - green; 2005 - orange; 2010 - red. A, B: stress = 14.9%, Axis 1 = 45%, Axis 2 = 25 %. C, D: stress = 16.8 %, Axis 1 = 38.2 %, Axis 2 = 38.1 %. E, F: stress = 13.61 %, Axis 1 = 46.7 %, Axis 2 = 26.2 %.

the study area. In fact, although globally relative humidity has remained constant (Held & Soden 2000; Sherwood & Meyer 2006), there is an increasing body of evidence supporting a significant upward trend in atmospheric water vapour (Willett *et al.* 2007). The fact that we are able to detect this trend prior to other classical climate metrics of temperature and precipitation, shows that it could work as an early warning metric of climate. On the other hand, our results show that lichen functional groups and composition were sensitive enough to detect these subtle changes. Firstly this highlights the importance of using ecological indicators to track climate change effects. If only classical metrics of climate were analysed, no changes would have been detected. On the other hand, our results highlight how powerful and sensitive lichens can be as ecological indicators of climate shifts. They were able to detect these subtle changes, not yet detected by classical climate metric analysis, and probably not by other less sensitive components of the ecosystems. Thus, their potential as early warning ecological indicators of climate change should be further explored in the future.

The large unidirectional change observed between 2005 and 2010, was preceded by back and forth transitions in lichen community composition between 1994 and 2005. Future work continuing this monitoring measures at regular time intervals is crucial to understand what part of this unidirectional change remains constant in response to climate shifts and what part can be attributed to normal population dynamics. This work is also essential for a higher confidence in attributing climate change as the cause of these changes.

Here, we observed that in the course of ten years no significant changes in temperature and precipitation were observed in the study area, but the number of days with relative humidity higher than 95 % has tripled over this time interval. It would be important to understand the origin of this shift, as in mainland Spain a downward trend has been observed since 1961 (Vicente-Serrano *et al.* 2014). A large dam was built approximately 100 km north of the study area, and it started to be filled in 2002. After completely full (in 2006), this became the biggest artificial water reservoir in Europe, so this could be the origin of the shifts in relative humidity. Nonetheless, to our knowledge, there is no information on whether or not this trend in relative humidity is generalized, nor if it results from the dam filling or of other atmospheric processes. The only certainty in terms of climate is that the study area is included in a region particularly susceptible to desertification and land degradation, due to an increasing aridity over the recent decades (Costa, Santos & Pinto 2012). Future works should focus on a better characterization of these climate shifts and its origin.

conclusions

Our results validate lichen functional diversity as a sensitive ecological indicator of climate change. Lichens have already been outlined as sensitive indicators of climate due to their fine scale responses to seasonal and range variations of temperature and precipitation, as well to mean relative humidity, and this work confirms its use over time. Lichen community composition changed significantly over the course of fifteen years (1994 - 2010), and these shifts were strongly related to subtle changes in climate. The compositional changes observed were mediated by changes in lichen functional composition, but not by species richness. This highlights that species richness is not enough, and metrics including abundance, community structure and functional diversity should be also contemplated when tracking the effects of climate change. Furthermore, our results indicate lichens ability to respond to subtle changes in climate not yet depicted by the currently used climate metrics. This suggests that they might have the potential to be used as early warning indicators of classic climate metrics related to temperature and precipitation, so future work should be devoted to explore this potential and to understand if they are able to signal these changes before other less sensitive components of the ecosystems.

acknowledgments

This work was supported by FCT-MEC through: project PTDC/AAG-GLO/0045/2014; P.M. by contract SFRH/BD/51419/2011; P.P. by contract SFRH/BPD/75425/2010; A.N. by contract SFRH/BD/51407/2011; C.B. by contract Investigador FCT. We acknowledge SOMINCOR (Sociedade Mineira de Neves Corvo, SA) for the financial support to this work. We thank IPMA (Instituto Português do Mar e da Atmosfera) for granting us access to climate data.

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chapter seven

General discussion

Growing evidence shows us that climate has changed in the recent decades (IPCC 2014). Global warming and changes in precipitation patterns worldwide, have impacted natural and human systems, and even if all greenhouse gas emissions stop, the scenario for the future will most likely worsen (IPCC 2014). A set of essential climate variables is being developed to keep track of on-going climate change (GCOS 2004). However, although this is an essential measure for attribution of causes, better predictions and to define risk assessments, vulnerabilities and adaptation measures, this is not enough to monitor climate change effects on ecosystems. It is imperative to understand and quantify how ecosystems functioning are affected by and respond to these changes (a 2ºC change does not have the same effect in alpine and desert ecosystems). For that we need universal ecological indicators preferentially based on biodiversity metrics and capable of evaluating the effects of global change on ecosystems structure and function (Pereira *et al.* 2013).

The objective of this work was to develop lichen functional diversity as an integrated and global ecological indicator of climate change. This was based on the assumptions that lichens would be good ecological indicators of climate change and that space is a good proxy of time. We demonstrated that lichen functional diversity metrics can be used as ecological indicators of climate change, as we first assumed. To build the ecological indicators we posed several questions. Our first question was related to the lichen diversity sampling methodology. Two methodologies are currently used at the continental scale, US and EU, and we wanted to investigate if data derived from them could be used jointly under a trend analysis perspective. We found that in fact they can. Hereafter, retrospective and future trend analysis can be done using the framework developed in this work. Afterwards we wanted to know if lichen traits respond specifically to climate. We found that simple lichen traits related to main type of photobiont, growth form and size do respond to climate, namely to the range and seasonal variation of temperature and precipitation, and to relative humidity. However, the response was specific for each functional group, showing that functional groups belonging to the same trait do not share the same response to climate variables. Then, we wanted to understand if this relation with the underlying climate variables is as good and predictable as hypothesized in functional ecology. We confirmed that functional groups-based lichen metrics reflected the underlying climate variables in a predictable way, confirming that they can be used as ecological indicators of climate change. Once our approach was not limited by species identity, we wanted to know if this ecological indicator was potentially universal. We found that though lichens' response mechanism was transversal and common to all functional groups, unlike expected, each couple of functional group and

underlying climatic variable cannot be applied at the global scale; the response was specific to areas with the same climatic limiting factor (ex: water availability). So, taken this into consideration, how should these ecological indicators be used? Because lichen functional groups-based indicators respond to the underlying most limiting climate factors, and these vary as we move in the globe, its application must be stratified to the limiting factors. Finally, we wanted to understand if the space substitution assumed by us was valid over time. We validated this space-for-time approach, and found that the ecological indicators developed for a specific climate were in fact those responding to climate shifts over fifteen years. More importantly, lichen trait-based indicators were able to respond to shifts in climate no yet depicted by classic climate metrics of temperature and precipitation (like its annual range or seasonal variation over the same period). Thus lichens are in fact excellent ecological indicators to track the effects of climate change, and have the potential to work as early warning indicators of classic climate metrics. These results have a highly important practical application. International organizations such as the UN three sister conventions have long demanded for a set of ecological indicators to track the effects of climate change on ecosystems, to be monitored continuously over time. From now on, lichens can be included in this set of ecological indicators to track the effects of climate change.

starting from scratch: building a universal sampling framework

A framework was developed and tested to compare data generated in Europe and North America (chapter two, Fig. 5). Our work was the first direct comparison of results from EU and US methodologies, which are currently the most widely used protocols at a continental scale: Europe and North America (Asta *et al.* 2002; USDA 2011). This was made featuring a paired dataset collected with both methodologies at the same sampling sites in the US Pacific northwest, following climate and nitrogen pollution gradients. Because measuring the multidimensional nature of biodiversity change calls for several metrics (Balmford, Green & Jenkins 2003; Pereira *et al.* 2010), the framework designed contemplated three different measures: taxonomic diversity (species richness, Shannon's and Simpson diversity indices), community structure and functional diversity (CWM of traits related to nitrogen tolerance). Methodologies yielded highly similar trends for all these metrics in response to nitrogen pollution and climate gradients.

Our framework sets the basis for future cross-continental analysis of lichen biodiversity trends from North America and Europe in response to global change. A global biodiversity monitoring network is set in motion to assess and keep track on the effects of global change (Pereira & Cooper 2006; Scholes *et al.* 2012).

This work has taken us one step closer to the inclusion of lichens in this network. Lichen diversity data now complies with the essential requirements to be included in this network, as data collection is based on compatible standardized methodologies performed at regional and global scales (Mace *et al.* 2005; MEA 2005; Pereira & Cooper 2006). A good place to start monitoring lichen diversity is the international LTER network. These are long-term research sites spread along key ecosystems at a worldwide scale devoted to monitor global change effects on ecosystems. The Portuguese Montado LTER already contemplates a protocol for lichen monitoring (http://www.ltsermontado.pt). We should now gather efforts to try also to include lichen diversity monitoring in the other LTER sites worldwide, as tool to track the effects of climate change and other global change drivers on ecosystems.

do lichen traits respond to climate? as a whole, or is the response specific to the functional group?

As expected, lichens responded to climate and a set of simple traits mediating this response was identified (chapters three and four): main type of photobiont, growth form and a coarse size classification. However, the response was not common to all functional groups belonging to the same trait, but was rather specific of the functional group considered (chapter four). This was the first comprehensive work on the main climatic variables acting on lichen trait environmental filtering (chapter four). Overall, we found temperature related variables to be the key predictors of lichen traits and their respective functional groups' response along a Mediterranean climatic gradient. Interestingly, lichens response was not related to mean annual temperature, but rather to its range and seasonal variation. Similarly, the residual importance of precipitation on lichen trait filtering was also related with its seasonal variation, rather than its total amount. Lichens sensitivity to temperature reflects the control temperature and water exert on lichen physiology (Lange, Kilian & Ziegler 1986; Sundberg *et al.* 1999; Gauslaa 2014), suggesting that these factors may be also determining each functional group temperature niche. It would be interesting in the future to further investigate how these differential sensitivities reflect the different ecological niches of each functional group, and how their ecological niche size relates to their capacity to be good ecological indicators.

Lichen functional groups differential responses and their sensitivity to seasonal and range variations are very important under a climate change perspective. In southern and central Europe, summer and daytime extremes are expected to raise faster than mean temperature (Kirtman *et al.* 2013). On the other hand, summer and winter trends from 1950 to 2010 highlight differential seasonal patterns of change, a trend that may continue in the near future (Kirtman *et al.* 2013). These observed

and projected trends in climate reinforce lichen potential to track these broad array of climatic variables forecasted to change.

do these trait-based metrics relate to the underlying climate variables in a predictable way?

The functional groups-based indices (namely community weighted mean, CWM) responded consistently in a predictable way (monotonically) to climate along spatial gradients as hypothesized in functional ecology, so they can be used as ecological indicators (chapter 5). Previous works suggested that the relations between trait-based indices and environmental variables are more likely to be consistently monotonic and predictable than those of species richness (Cornwell, Schwilk & Ackerly 2006; Mouillot *et al.* 2012). Although we were also able to find predictable monotonic relations between climate variables and species richness along climate gradients in several parts of the world, our trait-based indices resulted in general better predictors (Fig. 1). Relying solely on species richness may have other disadvantages, like its low informative power, and its lack of responsiveness or universality. For example, we found species richness to have the best relation with climate variables along an altitudinal gradient in a wet temperate climate (Fig. 1). This informs us about a decline in species richness with increasing altitude, probably as a result of the known species-energy relationship that explains the large-scale species richness patterns over the globe (Hawkins *et al.* 2003). However, no information is given about the species shifts that are occurring in the ecosystem. As it has been progressively demonstrated, to have an integrated picture we need a functional approach (Díaz & Cabido 1997; Díaz *et al.* 2007; Lavorel *et al.* 2007; Suding *et al.* 2008; Mouillot *et al.* 2012). Functional diversity responds faster and quantifies better ecosystems response to global change, providing simultaneously a strong link with ecosystems functioning and their services provision, independently of species richness reactiveness.

Furthermore, besides being responsive, the traits used in our work are easily identifiable, adding the advantage of requiring minimum expert knowledge to be identified. This was done with the purpose of ensuring its straightforward application in regions of the world where lichen flora is poorly known, guaranteeing that way the application of these ecological indicators independently of species identity. An example is given in figure 1 using results from different parts of the world, and with decreasing levels of knowledge on species: i) species identity and all information on traits available; ii) species identity and some trait information; iii) scattered information on species identity and just basic trait information available requiring minimum expert knowledge (the example of growth form is given). Even if information on species identity is incomplete, it is still possible to use these

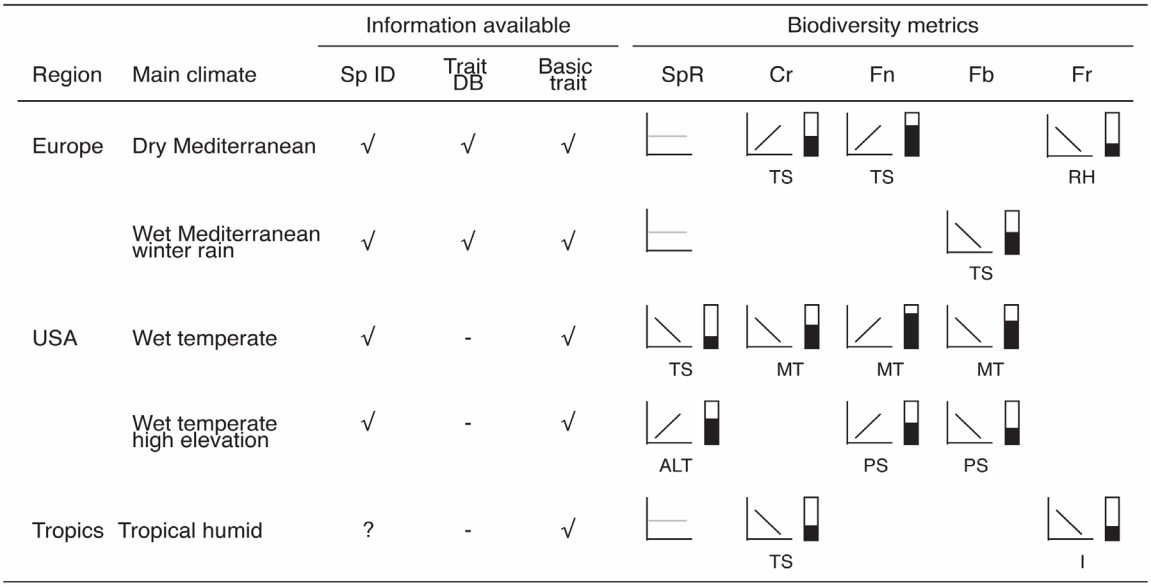

fig. 1. Conceptual framework on the information available to calculate lichen trait-based indicators in different parts of the world: Sp ID – species identifiable to the species level; trait DB – trait database available with complete information on major traits; basic trait – basic trait classification on main type of photobiont, growth form and size. Performance of taxonomic (species richness - SpR) versus functional diversity metrics (crustose – Cr, foliose narrow – Fn, Foliose broad – Fb, fruticose – Fr) in different parts of the world is also shown. Line graphs show modelled linear relationships (least squares multiple linear regressions) between biodiversity metrics and its best climate predictors: TS – temperature seasonality; RH – relative humidity; MT – maximum temperature; ALT – altitude; PS – precipitation seasonality; I - isothermality. Non-significant relations are depicted in grey. Bars after graph represent model fit (r^2).

indicators. Because the selected traits are so simple to identify, and because functional traits are independent of species identity and not bounded to species limited geographical distribution, its application is potentially more global.

can these ecological indicators independent of species identity be potentially universal?

Our results showed that the response mechanism is transversal and common to all functional groups, but unlike expected, there is no unique combination of functional group and climate variables that can be applied at a global scale (chapter 5). Lichens functional groups responded directly to the climatic limiting factor, and this mechanism of response was universal for all the traits and functional groups considered. However, as functional groups responded to the limiting factors, and these vary spatially, its response also varied.

The complex and diverse limits imposed by the interaction of temperature, water and radiation on vegetation are well recognized (Churkina & Running 1998). In fact, a decade ago a work revealed clearly how the percentage of limitation imposed by each of these factors changes at the global scale (Fig. 2 A) (Nemani *et al.* 2003). For this reason, while in a dry Mediterranean climate cyanolichens may be good ecological indicators of shifts in the amount of precipitation or its seasonality, in a temperate climate where water is no longer limiting, this indicator may in turn signal changes in temperature seasonality or isothermality.

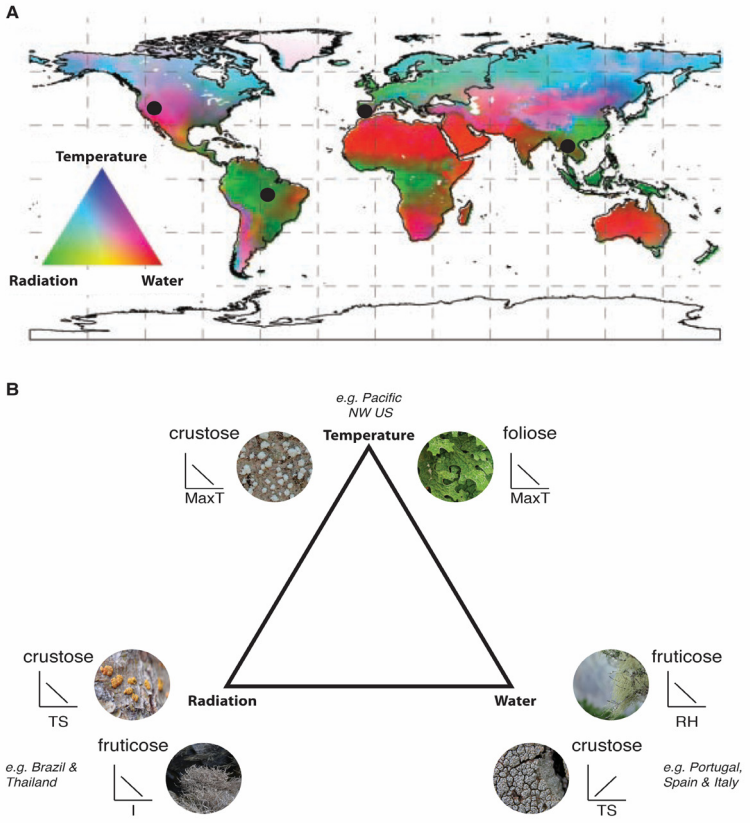

fig. 1. Geographic distribution of potential climatic constraints to plant growth derived from long-term climate statistics adapted from (Nemani et al. 2003) (A). Figure (B) illustrates conceptually how functional groups respond differently following these limiting factors as we move along this triangle moving from places were water is limiting (e.g Mediterranean), to places were temperature is more limiting (e.g. Pacific NW US) and finally to the tropics were radiation is the most limiting factor (here combining data from Brazil and Thailand). Black circles in figure A locate the sites were data shown in figure B come from.

Rather than a setback on its global application, this shows that these ecological indicators response should be interpreted alight of the new combination of limiting factors as we move in space, and the adequacy of the indicator to this new combination of limiting factors should be considered.

how should lichen functional groups-based indicators be used?

Lichen trait-based ecological indicators can be applied globally but because they respond to the most limiting climate factors, and these vary as we move in the globe, its application must be stratified by homogenous areas in terms of climatic limiting factors (chapter 5). It has been suggested that smaller spatial scales should be considered when working with ecological indicators based on species abundances along spatial environmental gradients (Hiddink & Kaiser 2005). This is related with Liebig's law (1840) that says that the limiting factor is the one that is least available at a particular time. As species abundance can be limited by several factors, if large spatial gradients are considered, chances are that more limiting factors act simultaneously making it more difficult to find an explanation for the spatial patterns of the ecological indicator (Hiddink & Kaiser 2005). In fact, when we divided the study area in climates with distinct limiting factors, the ecological indicators' response improved. An example is given

in Fig. 2 to illustrating how functional groups respond differently following the different limiting factors as we move on earth. Thus, the selection and interpretation of the lichen functional groups-based indicators must be done accordingly, following the different combinations of limiting factors specific for each climate.

can space be a substitute of time?

Our work validated space as a substitute for time. Space-for-time substitutions are based on the assumption that ecosystems will change in response to climate over time the same way they vary over space presently (Dunne *et al.* 2004). In this work, we used a temporal dataset of fifteen years, collected at 5-year intervals to test our trait-based ecological indicators developed along space. The ecological indicators signalling the climate shifts that occurred during these fifteen years, were those expected to do so according to our spatial predictions.

This validation over time was also important to reinforce our choice of functional diversity over taxonomic biodiversity metrics. While our lichen trait-based indicators were able to respond to subtle shifts in climate, species richness over the same period remained unresponsive. At local scales, species richness unresponsiveness was already shown for several organisms throughout various world ecosystems (Dornelas *et al.* 2014), regardless of the species decline expected globally due to climate change (Thomas *et al.* 2004). Additionally, it has been suggested that functional diversity metrics based on species traits and abundances, like those we used, can potentially work as early warning indicators because they respond faster and in a more predictable way (Villéger *et al.* 2010; Mouillot *et al.* 2012). Our results endorse these suggestions showing that in fact functional diversity metrics are more responsive and sensitive than taxonomic diversity metrics. Hence, our results show unambiguously that species richness is not enough to track climate change. Trait-based metrics are undoubtedly more responsive, and have a potential to improve biodiversity predictions under global change (Suding *et al.* 2008; Mouillot *et al.* 2012).

This work has also several implications under an early warning perspective. No detectable pattern of change was seen in classical metrics of temperature, precipitation (annual mean, range and seasonal patterns), and average relative humidity. However, after exploring other less commonly used climate metrics, we found a significant increase in the number of days with relative humidity higher than 95 %. Lichen functional groups-based indicators and composition were sensitive enough to detect these subtle changes. This illustrates the importance of using ecological indicators to track climate change effects, since if only classical

metrics of climate were analysed, no changes would have been detected. On the other hand, our results show how powerful and sensitive lichens can be as ecological indicators of climate shifts. Thus, their potential as early warning ecological indicators of climate change should be further explored in the future.

future challenges

Our work demonstrated how excellent lichens are as ecological indicators of climate change. Despite our current knowledge and the recent advances made thus far, the use of lichens as ecological indicators of climate change still has to overcome some challenges.

The next step should be to include them in the set of globally applicable ecological indicators long demanded by the United Nations Conventions on Biological Diversity (UNCBD), Climate Change (UNCCC) and to Combat Desertification (UNCCD). Monitoring these ecological indicators is fundamental to track the impacts of global change drivers (MEA 2005), to measure progress towards the targets set by these conventions, and to improve and guide new strategies for biodiversity conservation (Pereira & Cooper 2006) and for climate change mitigation and adaptation (Mace *et al.* 2005; MEA 2005). However, the small number of experts working with lichens worldwide may pose a limit to its global application. Therefore, an essential step for the future is to increase the number of available training courses to potentiate its application.

Because lichen communities aren't static over time, and may fluctuate in response to other biotic and abiotic environmental factors, future work should explore longer temporal datasets to more comprehensively understand short-term and long-term responses, as both are necessary to better predict the effects of climate change (Dunne *et al.* 2004).

There is a wealth of spatial and temporal information at local and regional levels in Europe and North America, collected by two standard methodologies that can hereafter be jointly analysed. Future work should focus on making trend analysis at higher spatial scales so that we can better understand lichen global patterns of change.

We've supported the need to incorporate metrics of functional diversity to allow a better quantification of climate change effects and to ensure a correct scaling up from local, to regional and global scales. However, some lichen traits used in this work are known also to respond to microclimatic conditions (Lakatos, Rascher & Büdel 2006; Pinho, Máguas & Branquinho 2010). Future work should focus on unveiling these relationships with microclimate, and to understand to which extent can such responses affect its performance as macroclimate indicators.

We've shown that cyanolichens can be clearly separated into two different groups: one more bounded to wet conditions and another one more resistant to dry conditions. While initially we thought that the resistant cyanolichens could be easily identified as being the jelly ones (with an homoiomerous internal structure), in chapter four we clearly observed that this is not the trait that separates them. Further work should try to unfold what physiological, structural or other kind of traits may underlie these lichens resistance.

Furthermore, we should also explore the trade-offs between using these simple traits versus the detail gained when using other more complex traits. Trait variation on lichen thickness, chemistry, or ratio alga/fungi biomass can be interesting to explore. Some of these traits have demonstrated links to changes in environmental factors, but they have seldom been explored under a climate change perspective (Sundberg *et al.* 1999; Gauslaa & Coxson 2011; Concostrina-Zubiri *et al.* 2014). This could not only be the basis for the development of other more complex ecological indicators, but it could also explain some of the patterns seen in the simple traits used in our work.

Genotypic variation related to traits may also be interesting to explore under a climate change perspective. Geographic genotypic variation in algal partners is known for lichen species, highlighting their dynamic nature (Yahr, Vilgalys & DePriest 2006). It would be interesting in the future to know if this dynamic nature of lichen symbiosis can play a role in climate change and if it can be used as an indicator as well.

Finally, climate change may arise in the future as one of the most pressing drivers of change (Steffen *et al.* 2015). Nonetheless, climate change will rarely act isolated. Effects of the interaction between climate change and other global change drivers on lichens, like pollution or land use, remain largely unknown (Branquinho, Matos & Pinho 2015). Thus, future research should contemplate the combined effects of major drivers of change so we can understand its combined effects.

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